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ABSTRACT VOLUME

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FRONTIERS UNBOUND: EXPLORING EXTREME ENVIRONMENTS

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FRONTIERS UNBOUND
 exploring extreme environments

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Emil G. Racoviță
Institute for the Study of Life in Extreme Conditions
Institutul pentru Studiul Vieții în Condiții Extreme



EMIL G. RACOVIȚĂ FOUNDATION
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Hadean recipes

Stephen J. Mojzsis

*HUN-REN Research Centre for Astronomy and Earth Sciences (CSFK) MTA Centre of Excellence
Konkoly-Thege Miklós út 15-17, 1121 Budapest, Hungary, stephen.mojzsis@csfk.org*

Key words: *Hadean Eon, prebiotic chemistry, planetary differentiation, hydrothermal systems, emergent land masses*

Life on Earth emerged at the dynamic interface of its primordial geosphere, hydrosphere and atmosphere. Silicate+metal worlds like Earth initially form hot via gravitational heating from accretion and differentiation, supplemented by decay of short- and long-lived radionuclides. Concurrent cooling sets off a chemical and mechanical cascade wherein siderophile elements (principally Fe+Ni) form a metallic core, and lithophile elements (Mg, Si, Al, Ca, Na, etc.) partition into a silicate mantle and crust. The outcome is an internally differentiated planet with a rocky surface beneath an outgassed fluid envelope of atmophile elements and compounds (CO₂, H₂O, H₂, noble gasses, etc.). In its first 500 Myr (*q.v.* Hadean Eon) Earth's crust co-existed with liquid water; it was affected by late accretion bombardments by asteroids and comets and harbored chemically diverse hydrothermal systems. Active volcanism and inherent differential buoyancy of the crust mandates that scattered emergent land masses exist irrespective of different estimates for past ocean volumes. Hadean land surfaces with diverse subaerial and (sub-)aqueous environments had the potential to establish prebiotic chemical ingredients to reactive concentration. The inferred complexity for even the minimum biological entity probably means that an operative and persistent biochemistry is the most difficult developmental stage to reach. Soon after planet formation, Earth's surface had the capacity to host full-fledged (i.e., living) biochemistry, but direct proof is lacking. Life originated on our planet, but it is unknown whether the earliest Hadean environments were ideally suited, or just good enough, to accomplish the task.



**Adapting to cold extremes:
insights from Ice Age to present-day Antarctic and beyond**

Woolly mammoth genomic adaptations to the extreme climate shifts of the Ice Age

David Díez-del-Molino^{1,2,3}

¹ Centre for Palaeogenetics, Svante Arrhenius väg 20C, 10691 Stockholm, Sweden

² Department of Zoology, Stockholm University, 10691 Stockholm, Sweden

³ Department of Bioinformatics and Genetics, Swedish Museum of Natural History, Box 50007, 10405 Stockholm, Sweden diez.molino@gmail.com

Key words: genomic adaptation, woolly mammoth, ancient DNA, cold climate, Ice Age.

The woolly mammoth inhabited terrestrial environments in the high Arctic during glacial periods, indicating that it was uniquely adapted to life in extreme cold conditions. Thanks to technological advances in the last decade it's now possible to reconstruct high-quality ancient genomes, which can provide a tool to investigate the genetic basis of adaptations in extinct organisms. In a series of studies, we have sequenced and analyzed the complete genomes of dozens of woolly mammoths.

This has allowed us to identify genomic adaptations, including private mutations (Díez-del-Molino et al., 2023) and genomic deletions (van der Valk et al., 2022), in genes associated with the species' iconic look as well as those putatively associated with tolerance to extreme climates. For example, we find woolly mammoth private genomic adaptations in genes related to hair growth and follicle shape (AHNAK2, KRT8, CD34, DROSHA, and TP63) as well as coat color (LYST), with fat metabolism and storage (ACADM, TET1, ACAD10, APOB, EPM2A, RDH16, and SEC31B), and with body temperature regulation and thermal sensation (several genes in the TRPM, TRPV and TRPA families, SCN10A, and CES2). We have also sequenced the genome of an early woolly mammoth (ca. 700,000 years old; van der Valk et al., 2021) to obtain estimates of when these adaptations evolved. We find that, at the time of its origin, the woolly mammoth had already acquired a broad spectrum of positively selected genes, which amounts to 92% of the private mutations observed in Late Quaternary woolly mammoths. Interestingly, our results suggest that these phenotypes continued to evolve throughout the Late Pleistocene but through positive selection on divergent sets of genes. For example, we find that genes associated with adaptation to extremely cold environments such as hair growth (PRSS8, TCHH and KRTAP4-1) and fat metabolism (PXMP4 and ADRB2) evolved only during that period. Finally, we also identify additional genes that underwent comparatively recent positive selection, including multiple genes related to skeletal morphology and body size (CD44, ANO5, and HSPG2), as well as genes that may have contributed to the smaller ear size in Late Quaternary woolly mammoths (FLG, ILDR1, and CHR1) also suggested to be an adaptation to cold environments.

These results provide a glimpse into the wide catalogue of genomic adaptations of the woolly mammoth and how these evolved over time in response to the shifting climates of the Pleistocene.

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Efficient cold-active extremozyme for aldehydes monitoring

Necula-Petrareanu Georgiana¹, Lavin Paris², Alina Vasilescu³, Purcarea Cristina¹

¹ Institute of Biology Bucharest, 296 Splaiul Independentei, 060031 Bucharest, Romania

georgiana.petrareanu@ibiol.ro

² Laboratorio de Complejidad Microbiana y Ecología Funcional, Instituto Antofagasta, Universidad de Antofagasta, 601 Angamos Av., Antofagasta, Chile

³ International Centre of Biodynamics, 1B Intrarea Portocalelor, 060101, Bucharest, Romania

Key words: cold-active aldehyde dehydrogenase, Antarctica, biosensors

In search of a versatile and highly stable extremozyme that can efficiently catalyse aldehydes' oxidation for biotechnologies and biosensing applications, the *aldH* gene (1506 bp) from the Antarctic psychrotolerant *Flavobacterium PL002* strain was cloned and heterologously expressed in *Escherichia coli* (Necula-Petrareanu 2022). This novel cold-active recombinant aldehyde dehydrogenase (F-ALDH; EC1.2.1.5) was purified in one step by affinity chromatography based on the N-terminal appended His-tag. Primary structure analysis revealed specific residues of psychrophilic homologs. Functional characterization of this enzyme indicated a high thermostability at temperatures ranging between 4°C - 55°C, with 60% residual activity at 60°C (Necula-Petrareanu 2022). Catalysis of F-ALDH could be carried out in the presence of both NAD⁺ and NADP⁺ cofactors using a variable range of aliphatic and aromatic aldehydes, with optimal reaction at pH 9.5 when using 4-isopropilbenzaldehyde as substrate in the NAD⁺-dependent reaction (Necula-Petrareanu 2022). Kinetic parameters calculated in the presence of multiple substrates indicated high catalytic efficiency and low apparent affinity for acetaldehyde. The particular stability after lyophilisation, storage at low temperature (-20°C), and incubation with a series of carbon black (CB) nanoparticles of this cold-active ALDH are assets for future developing stable ALDH catalyst in various biotechnologies, while the high sensitivity to heavy metals and dithiocarbamate fungicides indicated a potent biocomponent in developing biosensors for agricultural applications (Titou 2018, Titou 2020).

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Antarctic glacier melting modelling distribution of bacterial community structure

Corina Itcus^{1,2*}, Constantin Marin³, Soon Gyu Hong⁴, Manuela E Sidoroff², Cristian Coman⁵ Cristina Purcarea¹

¹*Institute of Biology Bucharest of the Romanian Academy, Bucharest, Romania, corina.itcus@ibiol.ro*

²*National Institute of Research and Development for Biological Sciences, Bucharest, Romania*

³*Emil Racovita Institute of Speleology, Bucharest, Romania*

⁴*Korean Polar Research Institute, Division of Life Science, Incheon, Rep. of Korea*

⁵*Institute of Biological Research, Cluj Napoca, Romania*

Key words: *Antarctic microcosm, 16S rRNA, glacier melting, ice microbial community*

Glacier retreat is expected to have a significant impact on the microcosm embedded in Antarctic perennial ice. In this context, our research focused on investigating the bacterial diversity from glacier ice of King George Island, NW Antarctica, in relation with the spatial distribution and substrate geochemistry.

Physicochemical measurements of the samples showed a decreased mineral content and slightly alkaline pH with a reduced organic carbon content and a homogenous sodium bicarbonate type chemistry.

16S rRNA gene sequencing carried out on ice samples highlighted variations of bacterial diversity in relation with the type of habitat, location and geochemical characteristics. The habitats showed spatial variations regarding the identified phyla, with a dominance of *Pseudomonatoda* followed by *Bacteroidota*. High content of *Gamma*- and *Betaproteobacteria* characterized the ice microbial communities, with a major presence of *Acinetobacter* species in all the sampled glaciers.

Investigation of the distribution pattern of bacterial communities entrapped in ice in relation with the habitat geochemistry, contributes to unravel the impact of melting glaciers on the resilience of the microcosm.

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Cave ice microbiome – reservoir for new biomolecules in medicine and biotechnologies

Victoria Ioana Paun^{1*}, Corina Itcus¹, Carmen Mariana Chifiriuc², Paris Lavin³, Cristina Purcarea¹

¹ Institute of Biology Bucharest of the Romanian Academy, victoriaioanap@gmail.com, ioana.paun@ibiol.ro

² Research Institute of the University of Bucharest-ICUB, Bucharest, Romania

³ Facultad de Ciencias Del Mar y Recursos Biológicos, Universidad de Antofagasta, Chile

Key words: potentially active microbiome, perennial ice, antimicrobial resistance, antimicrobial activity, bacterial cave isolates

The physiology of cold-environment microbiomes shows promise for discovering new bioactive compounds, yet the antibiotic resistance and antimicrobial potential of bacteria from perennial ice caves are largely unexplored. Our study characterizes bacterial strains from a 13,000-year-old ice core chronosequence from Scarisoara Ice Cave, Romania. These 68 bacterial isolates, the first from a perennial ice deposit accumulated since the Late Glacial period, provide the first culture-based evidence of bacterial resistome and antimicrobial compound production in this icy environment.

Bacterial strains from 28 ice core samples were identified through 16S rRNA gene sequencing and tested for antibiotic susceptibility and antimicrobial activity using the Kirby-Bauer method. The isolates, belonging to *Actinobacteriota*, *Pseudomonatoda*, *Bacillota*, and *Bacteroidota*, exhibited extensive resistance to 28 antibiotics. Eleven strains tested for antimicrobial activity against 22 human pathogens showed potential for developing novel antimicrobial strategies (Paun et al., 2021).

The study of icy-habitat microbiomes, including microorganisms in underground ice, is expanding. We analyzed total and potentially active bacterial communities from Scarisoara Cave ice core using Illumina sequencing. The potentially active bacterial community deposited over the last 13,000 years includes both autotrophs and heterotrophs, suggesting diverse metabolic processes in this icy habitat (Paun et al., 2019).

The broad antimicrobial resistance and multidrug-resistant phenotypes in bacterial strains from the ice core offer insights into the evolution of environmental resistomes. These findings present opportunities for discovering new bioactive molecules and cold-active biocatalysts. Combining data on antimicrobial resistance, activity, and biochemical characterization with active bacterial communities supports the concept of ice caves as a novel reservoir of biomolecules with diverse industrial applications.

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The ecosystem of a remarkable glaciovolcanic cave at Mount Meager, British Columbia, Canada

Christian Stenner^{1*}, Kathleen Graham¹, Morgan L. Cable², Glyn Williams-Jones³, Jared J. Clance⁴, Jacob Shaffer⁴, Jill Mikucki⁴, Anna Szyrkiewicz⁵, Olivia Vinnes⁴, Michael Paton², Jeremy Nash²

¹ Alberta Speleological Society, Calgary, Alberta, Canada, cstenner@telus.net

² NASA Jet Propulsion Laboratory, California Institute of Technology, Pasadena, USA

³ Centre for Natural Hazards Research, Department of Earth Sciences, Simon Fraser University, BC, Canada

⁴ Department of Microbiology, The University of Tennessee, Knoxville, USA

⁵ Department of Earth, Environmental, and Planetary Sciences, The University of Tennessee, Knoxville, USA

Key words: glaciovolcanism, glacier, volcano, astrobiology, Enceladus

The Mount Meager Volcanic Complex (Qwelqwelústen) is a glaciated volcanic massif located in the Garibaldi Volcanic Belt, British Columbia, which last erupted ~2360 yr BP. In 2015 a novel glaciovolcanic cave system was detected in the Job Glacier at Qwelqwelústen. The cave was formed from thermal flux originating from subglacial fumaroles in the underlying edifice, combined with glacial ablation, which melted open channels through the ice to the glacier surface (Unnsteinsson, et al., 2024). The only known glaciovolcanic cave in Canada, it provides an access point into a rare subglacial realm with a unique ecosystem formed via interaction of a warm vapour plume containing sulfidic volcanic gases with the surrounding ice.

Leveraging glacial and speleological exploration techniques, specialized hybrid Self-Contained Breathing Apparatus (SCBA) equipment for life safety, LiDAR, and survey methods, the cave at MMVC was explored and mapped. The exploration revealed glaciovolcanic cave geomorphology, subglacial microclimate, fumarole temperature and gas concentrations, fumarole biogeochemistry, composition of subglacial rock deposits, and enabled testing of precursor robotic technology for space exploration. Subglacial fumarole-associated sediments were characterized for microbial diversity, functional potential, and biogeochemistry including measurements of nutrients, major ions, organic and inorganic carbon, and stable isotopes.

Within the multi-level cave system, a passage along the rock-ice margin connected two primary low temperature fumarolic areas. The subglacial atmosphere included a diverse range of volcanic gases, with H₂S >200 ppm, SO₂ >100 ppm, CO₂ ~5200 ppm, and CO ~230 ppm as measured maxima. Fumarole temperatures of 90°C along with multiple areas of heated sediments and the bedrock surface contributed to unstable ice seracs of the walls and ceiling. Visible near-infrared to short wavelength infrared (VNIR/SWIR) spectral analyses of subglacial samples revealed minerals associated with fumarolic and hydrothermal alteration. Metagenomic data from microbial communities isolated from cave sediments suggest diverse chemosynthetic lifestyles in the communities that utilize multiple dark CO₂ fixation pathways. Testing of the sensor head of the Exobiology Extant Life Surveyor (EELS), a NASA JPL mission concept, provided data for further development of the prototype's sensor capability.

Glaciovolcanism at Qwelqwelústen provides a terrestrial analogue feature for astrobiology and cryovolcanism; The release of fumarole vapors from subglacial cave systems could mimic plume eruptions on Enceladus, where constituents of sub-ice ocean or reservoirs may be transported to the outer surface, likely depositing material on the surface that might be accessible by spacecraft (Cable et al., 2021). Characterization of glaciovolcanic interactions at Qwelqwelústen supports the development of mission concepts and instrumentation applicable to future space missions. Further geobiological study of the site will improve understanding of subglacial microbial ecology and can provide insight on geophysical controls on the evolution of potential life beyond Earth.

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**Rock and life: ancient signatures
and modern insights from extreme environments**

Sampling and DNA analysis of extreme environments

Scott Tighe

¹ *Advanced Genomics Core Lab, Center for Biomedical Shared Resource, University of Vermont, Firestone Building 150B, Burlington Vermont, USA 05405, scott.tighe@uvm.edu*

² *Extreme Microbiome Project*

Key words: *Extreme Microbiome Project, International Space Station, Microbiome, next generation DNA sequencing, Nanopore Sequencing technologies*

The ability to perform advanced metagenomic techniques on samples collected from extreme environments demands high performance reagents and techniques not commonly used in most labs today. The Extreme Microbiome Project was established in 2015 to develop novel sampling and DNA sequencing protocols for profiling of extreme environments. Samples have been collected and sequenced from around the world including Greenland glaciers, Antarctic lakes, Romanian caves, thermophilic ecosystems, halophilic, acidic, and alkaline lakes, as well as organisms from the International Space Station (Tighe et al., 2017; Green et al., 2023).

Field techniques including the use of novel liquid concentrating devices such as the Innovaprep CP, sample disruptors and homogenizers such as handheld bead ruptors and biopulverizers for hard sample, and the portable uTitan automated DNA extraction system. Samples are extracted for DNA using DNA-free reagents and pre-digested with high performance lytic enzymes including Metapolyzyme and Exopolyzyme followed by freeze fracturing prior to routine bead beating DNA extraction. Exotic techniques for chemically encapsulated cells include solvent exchange techniques using xylene and ethanol prior to DNA extraction. For samples with remarkably low cell numbers and biomass, the use pre-amplification using multiple displacement amplification technology combined with Nanopore or Illumina sequencing have been employed. All microbiome, metagenomic, and assembled genome data are generated using a combination of both short and long read sequencing including Illumina, Singular G4, and Oxford Nanopore Technology.

Results have demonstrated that organisms isolated from psychrophilic, acidic, and alkaline environments are difficult to recover DNA regardless of pre-digestion, beater beads, and extraction kits (Tighe et al., 2024). Halophilic and thermophilic samples benefit from a pre-wash step and extended enzymatic digestion. Whole Genome DNA sequencing results indicate that for most microbiome samples encountered in the extreme microbiome project, 5-30% of all organisms are not classified beyond genus with a large number being new species. Ongoing efforts include developing additional microbial extraction enzyme cocktails for fungi as well as advanced DNA extraction technologies and DNA-free reagents for low biomass samples such as those encountered in space research and planetary protection applications.



Figure 1. Example of two sample matrix collected by the extreme microbiome project. Left: Toxic hot springs in the Danakil Depression in Ethiopia; Right: Glacial and icebergs in the Baffin Bay, Greenland.

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First biogeochemical study of Mira de Aire Cave: the largest karstic system in Portugal

Alba Gómez-Arias¹, Julio Castillo², Nicasio Jimenez-Morillo¹, Beatriz Cubero¹, Sara Gutierrez-Patricio¹, Pedro N. Jimenez-Morillo¹, Nuno Jorge³, José A. González-Pérez¹, José M. de la Rosa¹, Ana Z. Miller^{1*}

¹ Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS-CSIC), Seville, Spain, anamiller@irnas.csic.es

² University of the Free State, Bloemfontein, South Africa

³ Grutas de Mira de Aire, Mira de Aire, Leiria, Portugal

Key words: geomicrobiology, speleothems, biomineralization, Nanopore sequencing, analytical pyrolysis

This study attempts to elucidate the role of microorganisms in the biogeochemical cycle of elements in Mira de Aire, the largest Karstic system in Portugal. Speleothems and green biofilms have been analysed using Nanopore sequencing, confocal microscopy, field emission scanning electron microscopy with X-ray energy dispersive spectroscopy (FESEM-EDS), and evolved gas analysis mass spectrometry (EGA/MS) analytical pyrolysis.

The real-time 16S rRNA gene sequencing analyses demonstrate that speleothems are colonised mainly by the phyla Pseudomonadota and Cyanobacteriota. At the genus level, microbial dark matter represented by uncultured bacteria dominates this ecosystem, followed by members of the genera *Hyphomicrobium*, *Desmonostoc*, *Massilia*, *Pseudomonas*, *Nitrospira*, *Geobacter*. The inferred functionality suggests that the bacterial community hosts diverse metabolic pathways such as aerobic chemoheterotrophy, nitrate reduction, aromatic compound degradation, ureolysis, methylotrophy, and manganese respiration, among others. The bacterial network seems to be driven by *Aromatoleum*, *Nitrospira*, uncultured bacteria, and *Sphigomonas*, capable of carrying on all these metabolisms and acting as bridges contributing to the stability of the bacterial community (Fig. 1). Microscopy observations corroborate the presence of polymicrobial biofilms with filamentous structures and densely packed filamentary structures of different sizes, as well as spheric photosynthetic cells (Fig. 1). Biofilms are known to act as nucleation sites for mineral precipitation due to their variety of functional groups (Castillo et al., 2023). Newly formed bio-precipitates were observed on these filamentous structures surrounding carbonate and silicate minerals, which contain essential elements for the microbial ecosystem, including C, O, Ca, Mn, Mg, and Fe. At least three organic carbon (OC) pools were identified in most samples using EGA/MS, indicating varying levels of stability. Some samples were dominated by an OC pool released at 350°C, mainly consisting of aliphatic compounds, polysaccharides, and fatty acids. An intermediate OC compartment (around 400°C), was strongly influenced by non-specific aromatic compounds and lignin, suggesting that the organic fraction of these speleothems is influenced by surface plant biomass, likely introduced into the cave through leaching (Palma et al., 2024). A third OC compartment (at 500°C) is dominated by highly aromatic and alkyl-aromatic compounds. This suggests the presence of highly evolved organic matter due to microbial activity, resulting in highly stable organic compounds. These findings highlight the importance of studying the OC in speleothems to better understand their role in biogeochemical carbon cycles and climate change.

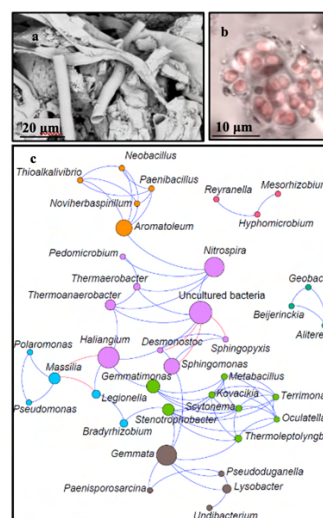


Figure 1. a) FESEM, b) CLSM, c) Bacterial network of speleothems from Mira de Aire Cave.

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Onsite microbial analyses in one of the most remote quartzite subterranean environment on Earth: Imawari Yeuta on Venezuelan tepuis

Martina Cappelletti^{1,2*}, Giacomo Broglia¹, Andrea Firrincieli³, Ettore Lopo¹, Daniele Ghezzi¹, Freddy Vergara^{2,4}, Francesco Sauro^{2,5}

¹ Department of Pharmacy and Biotechnology, University of Bologna, Bologna, Italy, martina.cappelletti2@unibo.it

² La Venta Geographic Explorations Association, Treviso, Italy

³ Department for Innovation in Biological, Agro-Food and Forest Systems, University of Tuscia, Viterbo, Italy

⁴ Teraphosa Exploring Team, Puerto Ordaz, Venezuela

⁵ Department of Geosciences, University of Padova, Italy

Key words: microbial diversity, MinION, DNA-based analyses, portable instruments, bioluminometer

Caves are extreme environments for life as they are characterized by the absence of light and poor supply of organic nutrients. Orthoquartzite caves in the table mountains tepui (in Venezuela and Brazil) are pristine, ancient and hard-to-reach subterranean environments. These caves host outstanding silica speleothems with stromatolite-like formations with different morphologies and in some cases huge sizes. In one of the orthoquartzite caves in Venezuelan tepuis, Imawari Yeuta, which represents the longest, and probably oldest known cave system in the quartzite subterranean scenery, previous morphological, geochemical and microbiological analyses demonstrated the presence of complex and abundant microbial communities which interact with the rock substrate leading to silica stromatolite formation (Sauro et al., 2018; Ghezzi et al., 2022).

In this work, we report the on-site analyses we conducted to study the microorganisms inhabiting these stromatolites and other signs of colonization on the quartzite rock. The analyses were conducted using portable instruments that allowed to have indications on the microbial activity level (using a portable ATP-detector) and to characterize the microbial communities (using Oxford Nanopore sequencing technology, ONT) present in different stages of silica stromatolite development and quartzite rock colonization. To do this, a temporary laboratory was installed in the cave (Figure 1), where the full DNA extraction, sequencing and data analysis procedure was conducted. A bioinformatic procedure was developed and applied in the cave laboratory to handle DNA sequencing data with limited resources and no internet connection. The results were statistically comparable with the data obtained in the laboratory using standard data analysis procedures, demonstrating the reliability of the information retrieved in the cave. The ATP- detector analyses were also confirmed in the laboratory by carrying out qPCR on the DNA that were extracted in the cave in the laboratory setting.

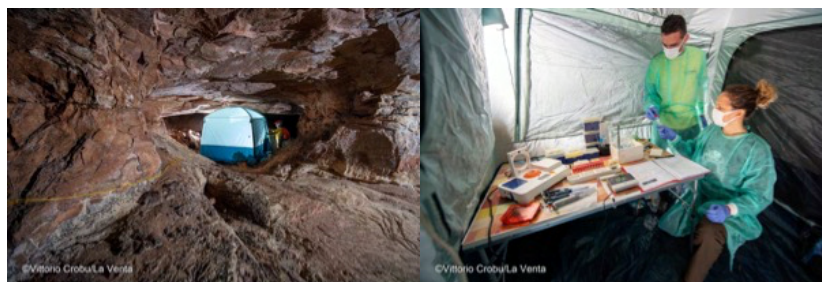


Figure 1. Pictures of the temporary laboratory installed inside Imawari Yeuta to perform *in situ* microbiological analyses (© Vittorio Crobu, LaVenta).

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Hraunrásir: Exploring and studying the steaming lava tubes of Fagradalsfjall Volcano (Iceland)

Francesco Sauro^{1,2,*}, Riccardo Pozzobon^{1,2}, Martina Cappelletti^{1,3}, Bogdan P. Onac⁴, Ana Z. Miller⁵, Gro Birkefeldt Mollen Pedersen⁶, Tommaso Santagata¹

¹ La Venta Geographic Explorations Association, Treviso, Italy, cescosauro@gmail.com

² Department of Geosciences, University of Padova, Italy

³ Department of Pharmacy and Biotechnology, University of Bologna, Bologna, Italy

⁴ University of South Florida, Tampa, USA

⁵ Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS-CSIC), Sevilla, Spain

⁶ University of Iceland, Reykjavik, Iceland

Key words: lava tube, eruption, metastable minerals, geomicrobiology

Fagradalsfjall Volcano erupted from March 19 to September 18, 2021 providing one of the most spectacular examples of shield volcano emplacement observed in recent times. During the eruption, it was possible to observe how lava tubes represent important flow pathways contributing to the build-up of the 4.8 km² lava field. The dataset collected during this eruption is unique thanks to the monitoring provided by webcam videos, photos, and repeated aerial photogrammetry surveys allowing reconstruction of tubes genesis. One year on from the eruption, lava tubes have become partially accessible, being in a transient cooling phase. The Hraunrásir project targeted this short window of time as a unique time to explore the tubes with the following objectives: 1) assess their morphology and the different mechanisms of lava tube genesis, 2) study their evolution during the cooling, 3) analyse which metastable secondary minerals form when the tube is still hot, and finally, 4) appraise how pioneer microbial life colonizes these originally pristine environments. All these topics have implications in the understanding of planetary lava tubes on Mars and other planetary bodies and their potential for astrobiology (Sauro et al., 2020). This project brought together a diverse team of researchers, both from Iceland and other countries, performing a total of five expeditions (October 2021, May 2022, October 2022, May 2023 and May 2024) on Fagradalsfjall allowing unprecedented observations of these newly formed subsurface environments. Here below we list some of the preliminary results: 1) the study of the tubes with different methods (magnetometry, 3D morphometry, etc.) has allowed to reconstruct the emplacement mechanism of Fagradalsfjall lava tubes; 2) spectacular metastable minerals have been sampled from different tubes at different temperatures with; 3) extremophile microbial life has been detected in at least two different cooling lava tubes, at temperatures ranging between 70° and 40° C. These bacteria represent the first colonizers of the extreme environment, showing that life quickly proliferates when T conditions allows (only 20 months after the eruption end), with implication for astrobiology.

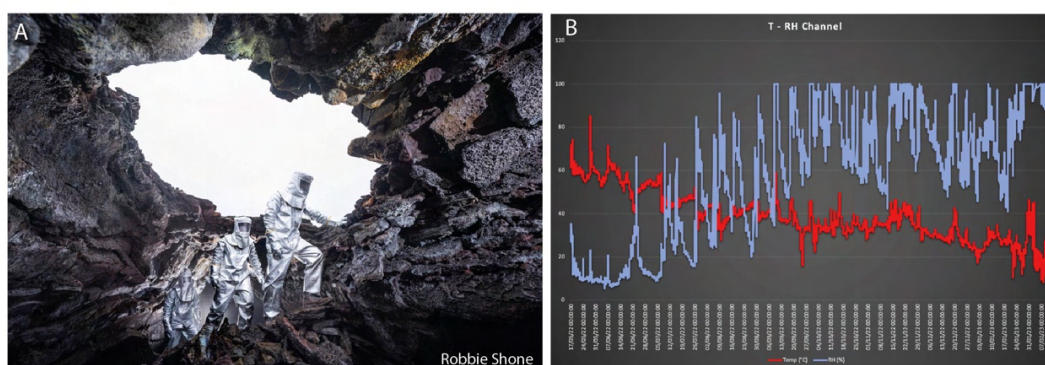


Figure 1. A) exploring a still hot lava tube in Fagradalsfjall (photo: Robbie Shone); B) Cooling temperatures and increasing humidity monitored in a lava tube for 18 months after the eruption.

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Human remains in now-extreme cave settings: exploring ancient funerary practices

Andrea Demjén¹, Florin Gogâltan², Szilárd Gall³, Călin Ghemiș⁴, Viorel-Traian Lascu⁵, Bogdan P. Onac^{6,7}, Ioana-Nicoleta Meleg^{7,8*}

¹ National Museum of Transylvanian History, C. Daicoviciu 2, 400020 Cluj-Napoca

² Institute of Archaeology and Art History, Kogălniceanu 12-14, 400084 Cluj-Napoca, Romania

³ Mureș County Museum, Str. Mărăști 8A, 540329 Târgu Mureș, Romania

⁴ Țării Crișurilor Museum, Calea Armatei Române 1A, 410087 Oradea, Romania

⁵ National Institute for Research and Development in Environmental Protection, 060031 Bucharest, Romania

⁶ School of Geosciences, University of South Florida, 4202 E. Fowler Ave., Tampa, 33620 USA

⁷ Emil G. Racoviță Institute, Babeș-Bolyai University, Clinicilor 5, 400006 Cluj-Napoca, Romania, ioana.meleg@ubbcluj.ro

⁸ Emil Racoviță Institute of Speleology of the Romanian Academy, Calea 13 Septembrie 13, 050731, Bucharest, Romania

Key words: cave, Bronze Age, funerary practices, Neolithic Age, NW Romania

The Izbucl Topliței de Vida Cave, situated in the karst system of the Răcaș Plateau within the Pădurea Craiului Mountains in northwest Romania, is renowned for its extreme environment. Accessing the cave is particularly challenging due to a former sump and a present-day 60-m-long lake with air-filled passages ranging from 5 to 50 cm. This unique setting has helped preserve its significant archaeological value. In the 1980s, researchers I. Emödi and G. Halasi conducted studies that uncovered several skeletal deposits and artefacts attributed to the Bronze Age, highlighting the cave's archaeological importance (Halasi et al., 1985).

Recent interdisciplinary research, initiated in 2021, has uncovered evidence of the cave's occupation during at least two distinct phases. Human remains dated to the Early Bronze Age (approximately 4500 cal BP) and contemporary funerary artefacts (e.g., ceramic vessels, gold ornaments, copper axes) were discovered alongside ceramic fragments, grains, and charred wood from the Middle Neolithic Age (around 7000 cal BP). Anthropological investigations revealed that most of these remains—belonging to males, females, infants, and adults—were in secondary position due to natural or human impacts and not found *in situ*. However, the few skeletons found *in situ* suggest that the cave served as a funerary or deposition site during the Early Bronze Age. Notably, while most of the bones did not show signs of violence and some skulls exhibited patterns consistent with disease, a significant exception was the beheaded remains of a child approximately 10 years old. This finding is particularly significant, as burials during this period were typically conducted within tumuli. The presence of disease markers and the isolated decapitated child offer valuable insights into the funerary practices and health conditions of individuals laid down in this subsurface environment.



This research highlights the cave's importance in understanding past human activities, providing a unique perspective on historical funerary practices and the lived experiences of individuals across several millennia.

Figure 1. A 7-millennium archive of evolution and culture (photo: Irinel Cîrlănaru).

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Insights into past extreme environments through ancient biomolecules

Ioana-Nicoleta Meleg^{1,2*}, Magdalena Năpăruș-Aljancic^{3,4}, Yuichi Naito⁵,
Hervé Bocherens^{6,7}, Axel Barlow⁸

¹ Emil G. Racoviță Institute, Babeș-Bolyai University, Clinicilor 5-7, 400006 Cluj-Napoca, Romania, ioana.meleg@ubbcluj.ro

² Emil Racoviță Institute of Speleology of the Romanian Academy, Calea 13 Septembrie 13, 050731, Bucharest, Romania

³ Karst Research Institute ZRC SAZU, Titov trg 2, SI-6230 Postojna, Slovenia

⁴ Tular Institute, Oldhamska 8A, SI-4000, Kranj, Slovenia

⁵ Central Research Institute of Electric Power Industry (CRIEPI), 1646 Abiko, Abiko-shi, Chiba 270-1194, Japan

⁶ Department of Geosciences, Biogeology, University of Tübingen, Hölderlinstraße 12, 72074, Tübingen, Germany

⁷ Senckenberg Centre for Human Evolution and Palaeoenvironment SHEP at the University of Tübingen, Sigwartstraße 10, 72076 Tübingen, Germany

⁸ School of Environmental and Natural Sciences, Bangor University, Bangor, LL57 2DG UK

Key words: ancient DNA, cave bears, stable isotopes, Romanian Carpathians

During the Pleistocene, from 2.6 million to 11,500 years ago, Earth's climate experienced extreme fluctuations, marked by glacial and interglacial cycles. These shifts forced species into refugia, affecting their genetic diversity and distribution. The period's end, compounded by human expansion, led to the decline and extinction of large mammals like the woolly mammoth and cave bear (Hewitt, 2000). Today, the study of ancient biomolecules, including aDNA and stable isotopes, is revolutionizing our understanding of Earth's history, revealing insights into species' ecology during extreme environmental changes.

The cave bear, an emblematic Pleistocene species that went extinct around 25,000 years ago, has extensive fossil remains across Eurasia. It was the first species to have its paleodiet inferred from stable isotopes in bone collagen and the first outside permafrost to yield ancient DNA (aDNA) (Barlow et al., 2019; Bocherens, 2019). While stable isotope analysis generally suggests an herbivorous diet, some individuals from Romanian caves showed unusual isotopic signal that has been interpreted by some researchers as evidence of omnivory (Bocherens, 2019).

We present paleogenomic and paleodiet data for cave bears across the Carpathian region in Romania, which served as a unique biological and edaphic interface compared to other European regions during the last glacial period. This distinctive context led to peculiar isotopic signals in the bone collagen of the assessed individuals, resulting in dietary variations specific to some Romanian cave bears. Using amino acids ($\delta^{15}\text{N}$ values of glutamate and phenylalanine), we found that dietary variation among these cave bears still falls within the herbivorous trophic niche. Moreover, this variability was not associated with population structure, sex (as inferred from aDNA data), time period (as inferred from radiocarbon and molecular tip-dating), bioclimatic conditions (deduced from vegetation modeling maps, chronology, and available climate reconstructions), or location. Thus, dietary variation co-occurred across these potential explanatory variables, suggesting individual behavioral responses to the availability of food resources, potentially linked to changes in forest cover. These results provide compelling evidence of the flexible capacity and resilience of these individuals over their lifespan in the face of extreme environmental shifts. However, this flexibility was not enough to prevent their decline and eventual extinction. The interdisciplinary approach used in this study unlocks a deeper understanding of ecological processes in extreme environments, revealing details that would remain hidden if each type of evidence were examined separately.

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Exploring microbial diversity in extreme environments

Given to salt: Novel insights into the diversity of halophilic microorganisms and their functioning in hypersaline ecosystems

Aharon Oren¹

¹ Department of Plant and Environmental Sciences, The Institute of Life Sciences, The Hebrew University of Jerusalem, Edmond J. Safra Campus, Jerusalem 9190401, Israel, aharon.oren@mail.huji.ac.il

Key words: halophiles, salt, hypersaline environments, biodiversity

In the past five years, our understanding of the diversity of halophilic microorganisms (defined here as growing in environments with more than 100-150 g/L dissolved salts) and their distribution in hypersaline ecosystems worldwide has greatly increased (Oren, 1999). High-salt-adapted microorganisms are found in each of the three domains of life: Archaea, Bacteria, and Eukarya (Oren, 2011). Novel lineages of halophiles that are still awaiting to be isolated have been discovered by cultivation-independent approaches (small subunit rRNA gene libraries, metagenomics), and use of different cultivation methods resulted in the isolation of many novel types of halophiles in pure culture. Interesting cases of cooperation have been discovered involving different phylogenetic lineages, such as the symbiosis between members of the archaeal class *Halobacteria* and the ‘*Candidatus* Nanohalarchaeota’, Metagenomic approaches have taught us much about the intraspecies diversity of model organisms such as *Haloquadratum walsbyi*, and *Salinibacter ruber* (Oren, 2024). Cultivation-dependent approaches enabled the recognition of novel metabolic types not earlier reported from hypersaline ecosystems. Microbiological studies of previously unexplored environments have increased our understanding of the limits of life at high concentrations of chaotropic ions.

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Recent advances in the microbiology of permanently stratified, hypersaline lakes of Transylvanian Basin (Central Romania)

Horia L. Banciu^{1,2,*}, Ionuț M. Gridan¹, Adrian V. Zety¹, Ioana Drăghici¹, Cristina Mircea¹, Michalis D. Lazăr¹, Andreea M. Tripon¹, Doriana M. Buda¹, Adorján Cristea³

¹ Department of Molecular Biology and Biotechnology, Faculty of Biology and Geology, "Babeș-Bolyai" University, 400006 Cluj-Napoca, Romania, horia.banciu@ubbcluj.ro

² "Emil G. Racoviță" Institute, "Babeș-Bolyai" University, 400084 Cluj-Napoca, Romania

³ Department of Taxonomy and Ecology, Faculty of Biology and Geology, "Babeș-Bolyai" University, 400006 Cluj-Napoca, Romania

Key words: biogeochemical roles, halophiles, hypersaline lake, metagenomics, microbial communities

Present-day Romanian territory harbors several types of habitats classified as 'extreme environments', including hot springs, subterranean ice caps, and hypersaline lakes. Many of these habitats are in the Central and North-Western Romania, in or around the Carpathians. Among them, hypersaline waters (e.g. with salinity several times higher than that of the seawater, i.e. 3.5 g/L total salts) are prevalent in terms of size and incidence. Over 40 hypersaline lakes of various areas (~<0.1 to ~4 ha), shapes (irregular or bell-shaped), and origin (natural or anthropic) are found throughout Transylvanian Basin (Central Romania) making this region very attractive for the exploration of extremophiles, and, specifically, halophilic (salt-adapted) organisms.

Depending on their hydrology (e.g. input of freshwater) and morphometry (e.g., depth), Transylvanian hypersaline lakes can be categorized into shallow (a few meters depth) or deep (down to ~100 m depth), with the latter type showing permanent density stratification ('meromixis'). The meromictic lakes are natural laboratories allowing the assessment of changes in the structure, composition and ecological interactions of microbial communities along steady vertical physico-chemical gradients such as salinity, pH, redox potential, dissolved oxygen, or various nutrients (Alexe et al., 2018). Thus, considering the ecological distinctiveness as well as dominance of permanently stratified, hypersaline lakes in Transylvanian Basin, extensive efforts were focused on elucidating the microbial diversity and ecology over the past decade. Thanks to their peculiar abiotic characteristics, Ursu Lake (Sovata, Mureș County) and Fără Fund lakes (Ocna Sibiului, Sibiu County) were thoroughly surveyed as 'model' lakes for diversity and biogeochemical roles of microbial communities including bacteria, archaea, and fungi. While conventional cultivation approaches have provided limited scientific novelty, extensive microbiological understanding of Transylvanian meromictic lakes has been gained by next-generation sequencing - NGS (amplicon sequencing and omics-based) studies (Baricz et al., 2021; Viver et al., 2023). Briefly, it was revealed that despite their similar categorization as meromictic and hypersaline, Ursu and Fără Fund lakes have distinct vertical partitioning of microbial communities. The oxic and photic surface water masses of these lakes are inhabited by halotolerant *Bacteria* and halophilic *Archaea* members, respectively, a finding that could be reasonably explained by markedly different salinities (0-6% and 19-20%, respectively). The hypersaline, anoxic, and aphotic bottom waters of both lakes, however, seem to share microbiomes dominated by halophilic fermenters (e.g., *Halanaerobiales*) and sulfate reducers (e.g., *Desulfovibrionales*) (Baricz et al., 2021). Furthermore, the amplicon (18S, ITS2) sequencing-based analyses, have indicated that the two model lakes house distinct fungal communities (unpublished data). Additionally, the metagenomic and metatranscriptomic study of water column and sediments of Ursu and Fără Fund lakes, has indicated a microbiome diversity dominated by uncultured or candidate *Bacteria* and *Archaea* lineages (unpublished data) with yet-unknown roles in nutrient cycling and ecosystem functioning thus highlighting the stringent need to further explore these extreme environments.

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Prokaryotic inhabitants of hypersaline brines from former salt mine in Solivar (Presov, Slovakia)

Shivani Adhvaryu¹, Maria Piknova¹, Lea Nosalova¹, Lenka Malinovicova¹, Sonja Brestovicova¹, Jana Kiskova¹, Mariana Kolesarova¹, Peter Pristas¹

¹ Department of Microbiology, Institute of Biology and Ecology, Faculty of Science, Pavol Jozef Safarik University in Kosice, Srobarova 2, 04101 Kosice, Slovakia, peter.pristas@upjs.sk

Key words: salt brine, cultivable bacteria, halophiles, phylogeny, biotechnology

The study of microbiota inhabiting extreme hypersaline environments such as soda lakes, salt mines, or deep-sea salt brines has gained increasing interest in recent years. Such environments were traditionally considered as having low variability of bacteria, colonized mostly by highly adapted Archaea. However, it is now becoming clear that these extreme habitats harbour complex microbial communities with immense importance in various fields of biotechnology.

More than 200 cultivable halotolerant or halophilic heterotrophic isolates from hypersaline brine from Solivar near Presov (Slovakia) were obtained during 2020–2022 years and characterised. The season showed the most important impact on observed diversity of heterotrophic bacteria. The low diverse halophilic microbiota represented by Proteobacteria members (mainly *Halomonas*, *Halovibrio*, *Idiomarina*, and *Chromohalobacter* spp.) was replaced by more diverse population of halotolerant Firmicutes (mainly *Peribacillus*, *Rosellomorea*, and *Bacillus* spp.) probably due to different levels of precipitations. Genome analyses were used to identify the molecular mechanisms of adaptation to hypersaline oligotrophic environments. Nearly 80% of strains isolated were able to grow autotrophically using sulphur oxidation as a sole energy source. In the following series of experiments halophilic autotrophic sulphur oxidizers were found to be dominated by Proteobacteria members of *Guyparkeria* and *Hydrogenovibrio* genera. Surprisingly, the lowest diversity was observed for halophilic Euryarcheota when members of *Haladaptatus* genus were detected only.

Phylogenetic analyses indicate that nearly half of isolated strains could be representatives of new species and many isolates showed the potential for environmentally friendly applications in biodegradation, decolorization, food biotechnology, and biopolymers production.

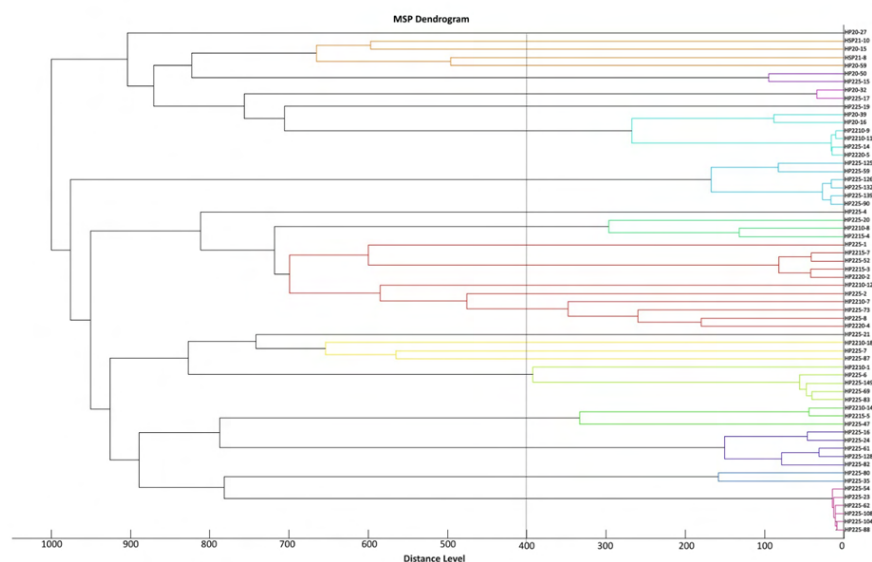


Figure 1. The diversity of isolates from hypersaline brine from Solivar, Presov as observed by comparison of whole cells protein profiles obtained by MALDI-TOF Mass Spectrometry.

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Exploring pristine microbial ecosystems from the uninhabited volcanic Selvagens Islands

Pedro N. Jiménez-Morillo¹, Sara Gutiérrez-Patricio¹, Vera Palma², Ana T. Caldeira², Nicasio T. Jiménez-Morillo¹, Ana Z. Miller^{1,2*}

¹ IRNAS-CSIC, Seville, Spain, anamiller@irnas.csic.es

² Hercules Lab, University of Évora, Évora, Portugal

Key words: Extreme environment, remote islands, next generation sequencing, Archaea, geomicrobiology, biogeochemistry

This study focuses on the microbial characterization of sedimentary cores extracted from the Selvagens Islands (Madeira, Portugal). Located in the North Atlantic, these volcanic islands form an archipelago consisting of two main islands that emerged during the Oligocene period (25-29 Ma). Renowned for their extraordinary marine and terrestrial biodiversity, these islands harbor many endemic species. Remarkably untouched by human activities, this isolated and undisturbed ecosystem offers an exciting opportunity to investigate preserved biosignatures within rock formations and sedimentary deposits. By analyzing the microbial communities in terrestrial sedimentary cores, we aim to gain insights into the microbial diversity, ecological roles, and survival strategies of these pristine ecosystems. Sediment samples were collected along a gradient on a small slope from *Baía das Galinhas*, on *Selvagem Grande* Island. Each sedimentary core was sliced into 2 cm wide sections. The most superficial and deepest layers of each core were subjected to a combination of microbiological techniques, including 16S rRNA gene sequencing, metagenomics, and culturing methods, to identify and understand the role of microbial populations present in each sediment core. The DNA-based sequencing analysis revealed a clear predominance of microorganisms belonging to the domain Archaea in all sediment samples, with the phylum *Euryarchaeota* being the most abundant in all samples. The bacterial phyla *Actinomycetota*, *Pseudomonadota*, and *Bacillota* were also found to a lesser extent. Archaea can thrive in extreme environments such as hot springs, terrestrial and marine sediments, hydrothermal vents, saline lakes, permafrost soils, and siliceous speleothems, where they play essential ecological roles. In this study, we also inferred the functional capabilities of the microbial communities. First, we observed that the most abundant predicted pathways in most sediment samples are related to aromatic degradation compounds and chemoheterotrophy. These pathways indicate the capability of microbial metabolism to decompose complex compounds and utilize organic compounds as a source of energy and carbon. On the other hand, we found metabolic pathways related to photosynthetic organisms, as phototrophy is one of the most abundant pathways in the most superficial core layers. This research provides valuable insights into the diversity and functionality of microorganisms in volcanic sediments, emphasizing their adaptability and ecological importance.

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Microbiome diversity in acidic environments: the case of the Sesei Valley tailing pond

Julia Lupan^{1,2,*}, Alexei Remizovschi¹, Rahela Carpa¹, Ferenc L. Forray³, Oana-Raluca Koblicska⁴, Călin Gabriel Floare⁵, Claudiu Tanaselia⁶, Octavian Popescu⁴

¹ Department of Molecular Biology and Biotechnology, Faculty of Biology and Geology, "Babeş-Bolyai" University, 400006 Cluj-Napoca, Romania, julia.lupan@ubbcluj.ro

² "Emil G. Racoviţă" Institute, "Babeş-Bolyai University, 400084 Cluj-Napoca, Romania;

³ Department of Geology, "Babeş-Bolyai" University, 400006 Cluj-Napoca, Romania.

⁴ Molecular Biology Center, Institute for Interdisciplinary Research in Bio-Nano-Sciences, Babes-Bolyai-University, 400084 Cluj-Napoca, Romania

⁵ National Institute for Research and Development of Isotopic and Molecular Technologies, Donath 67-103, 400293, Cluj-Napoca, Romania

⁶ Research Institute for Analytical Instrumentation Subsidiary, Donath 67, 400293 Cluj-Napoca, Romania

Key words: acidic mining drainage, acidophilic bacteria, microbial communities

Acid mining drainage and tailings ponds resulting from mining operations pose serious environmental problems due to low pH and high concentrations of heavy metals. These extreme ecosystems harbor unique microbiomes generally characterized by low diversity. Exploring these microbiomes is essential for the development of potential bioremediation strategies for these contaminated sites. These environments can be considered living laboratories that provide unique opportunities to study microbial communities under conditions from the past that no longer exist (Havig et al., 2017). Copper mining at Roşia Poieni has a history spanning almost half a century, and the tailings pond was built a few years after the mining activities started. The mining activity in this area has been reported in several studies as the main polluting factor of the Arieş River. As a result of pyrite alteration at low pH, the acid rock drainages have high metal concentrations including Al, Fe, Cu, Mn, Ba, Zn, Pb, and As (Levei et al., 2013).

Analysis of the prokaryote microbial community structure using next-generation sequencing techniques (16S variable region) revealed the presence of the dominant phyla as Proteobacteria and Patescibacteria in all water and sediment samples, while Actinobacteriota and Acidobacteriota were mainly found in most acidic samples.

The most common genera in the samples with lowest pH included *Acidocella*, *Terracidiphilus*, *Acidithrix*, *Acidisphaera*, and various uncultured acidophilic bacteria. The uncharacterized Archaea were predominant in all samples, whereas Halobacteriota and Thermoproteota were present in varying abundances. A uniform distribution pattern of archaeal phyla such as Halobacteriota, Thermoproteota, Micrarchaeota, Thermoplasmata, and Nanoarchaeota was observed in the most acidic water samples. The most abundant archaeal genera were *Micrarchaeum*, the uncharacterized Micrarchaeaceae family, Thermoplasmatales-GCA-000496135, *Nitrosotalea* and *Cuniculiplasma*.

The pH values are the main factor that modulates the diversity of the prokaryotic communities in the analyzed samples, with a decline in diversity observed as pH values decrease. The overall diversity of the sediment samples was found to be higher than that of the water samples. The samples with moderately low pH were grouped together, while the most acidic samples formed distinct clusters. This clustering is maintained regardless of the sample type (water or sediment) and the type of microorganisms analyzed (bacteria or Archaea).

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**From high peaks to hidden depths:
ecosystem dynamics and environmental challenges**

High mountain ecosystems and snow cover in a changing climate: for better or for worse

Philippe Choler

Univ. Grenoble Alpes, Univ. Savoie Mont Blanc, CNRS, LECA, 38000 Grenoble, France
philippe.choler@univ-grenoble-alpes.fr

Key words: *alpine ecology, seasonal snow cover, climate change*

Snow cover duration is a critical driver of alpine plant distribution and ecosystem functioning. With climate change, both the extent and duration of the winter snowpack have significantly declined, a trend that has intensified in recent decades. Due to their strict dependence on prolonged seasonal snow cover, late snow-melting ecosystems, known as snowbeds, are key indicators of climate change (Choler et al., 2018). The growth and reproduction of vascular plants in these 'time-limited' habitats are severely constrained, and snowbeds support a unique taxonomic and functional biodiversity at the edge of vascular plant life (Choler et al., 2021). In my talk, I will present findings and insights on the dynamics of these ecosystems in response to climate change. I will highlight the balance between the gain of new habitats where (semi-)permanent snowfields disappear and the loss of habitats where snowbed specialists are outcompeted by mesic species (Choler et al., *in press*). I will also show the value of intersecting plant surveys with remote sensing techniques to enhance our understanding of these dynamics on both local and continental scales.



Figure 1. Early summer alpine landscape in the SW Alps showing late snow-melting sites (© Ph. Choler, CNRS).

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Deciphering above-treeline vegetation greenness trends: Insights from the Carpathian Mountains

Pavel Dan Turtureanu^{1,5*}, Arthur Bayle², Nicoud Baptiste², Olimpiu Traian Pop³, Mihai Pușcaș^{1,4,5}, Philippe Choler²

¹ A. Borza Botanic Garden, Babeș-Bolyai University, Cluj-Napoca, Romania, pavel.turtureanu@ubbcluj.ro

² CNRS, Laboratoire d'Ecologie Alpine, Univ. Grenoble Alpes, Univ. Savoie Mont Blanc, Grenoble, France

³ Faculty of Geography, Babeș-Bolyai University, Cluj-Napoca, Romania

⁴ Faculty of Biology and Geology, Babeș-Bolyai University, Cluj-Napoca, Romania

⁵ Emil G. Racoviță Institute, Babeș-Bolyai University, Cluj-Napoca, Romania

Key words: *alpine, greening, mountains, remote sensing*

Above-treeline ecosystems have experienced rapid warming and land use changes in recent decades, resulting in diverse vegetation responses, most notably an increase in vegetation cover. A growing body of research indicates that Arctic and alpine regions have undergone significant vegetation greening. The Carpathian Mountains, largely underexplored in ecological studies, offer a valuable opportunity to investigate these dynamics. In this presentation, we will discuss the trajectories of vegetation change over the past few decades. By integrating advanced remote sensing techniques with dendroecological surveys, we present the first comprehensive assessment of greening trends in this region. First, we conducted a high-resolution land cover classification using a training dataset created in Google Earth, applying a Random Forest algorithm to Sentinel-2 imagery. The land cover types included scree, grasslands, shrublands of Ericaceae species, *Pinus mugo*, *Juniperus communis* var. *saxatilis*, and *Picea abies* forest stands. Multiple spectral indices were used as predictors, including the Normalized Anthocyanin Reflectance Index (NARI) to distinguish the late-season spectral signature of Ericaceae-dominated shrublands. Greening was defined as the increase in the yearly maximum kernel Normalized Difference Vegetation Index (kernel NDVI) derived from Landsat images over the past 40 years at a 30 m resolution, using procedures that account for temporal autocorrelation in the NDVI time series. Our findings revealed widespread greening trends across the entire range. Additionally, areas classified as Ericaceae shrublands exhibited significantly higher greening trends compared to other land cover types. This was even more prominently highlighted in the Partial Dependency Plots, which showed accelerated greening trends at higher probabilities of Ericaceae land cover. By comparing several instances in the available historical Corona spy satellite images from the 1960s with recent high-resolution aerial images from Google Earth for changes in woody vegetation dominance and signs of shrub recruitment, we believe that the ecological processes driving this pattern are the infilling / increase in Ericaceae cover. Additionally, field dendrochronology observations suggest that, in some cases, this may be accompanied by a recolonization of shrub species (Fig. 1). Overall, the observed vegetation changes were likely driven by a combination of reduced grazing pressure and climate warming, leading to shifts in ecosystem structure and functioning. Our research not only underscores the dynamic nature of these high-elevation ecosystems, which might otherwise be considered relatively stable, but also emphasizes the critical role of interdisciplinary approaches in deciphering the ecological mechanisms underlying greening in high-altitude environments.

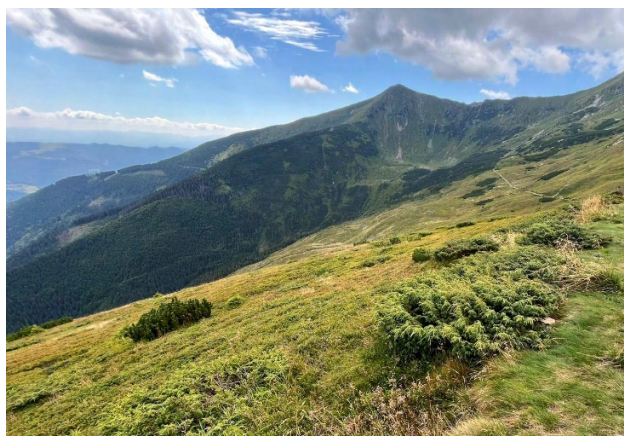


Fig. 1. Mountain slope in the Rodnei Mountains dominated by Ericaceae, with scattered individuals of Pinus mugo and Juniperus communis var. saxatilis.

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The ecology of chemosynthesis in submerged coastal caves

Robert Scharping^{1,2,3*}, Michael Casso², Isabel Schaal³, Paul Henderson³, James Garey⁴,
Matthew Charette³, John Pohlman², Stefan Sievert³

¹ University of Florida, Gainesville, Florida, USA, rscharping@ufl.edu

² US Geological Survey, Woods Hole, Massachusetts, USA

³ Woods Hole Oceanographic Institution, Woods Hole, Massachusetts, USA

⁴ University of South Florida, Tampa, Florida, USA

Key words: Nutrient cycling, groundwater, submarine spring, chemolithoautotrophy

Carbonate coastlines account for ~16% of coasts worldwide. Karst subterranean estuaries (KSEs) form along carbonate coasts where seaward-flowing groundwater interacts with landward-intruding seawater, and these ecosystems significantly modify aquifer exports of carbon and nutrients. It is currently not well constrained how some subsurface microbial processes, such as chemosynthesis, alter the elemental exports of KSEs. To fill this knowledge gap, we studied the microbial community and geochemistry of the Double Keyhole KSE (Florida, USA). The karst conduit associated with the Double Keyhole KSE expels brackish groundwater as a submarine spring and hosts several density-stratified groundwater masses. We found that the sharp interface between the two uppermost water masses exhibits opposing concentration gradients of dissolved oxygen and total dissolved iron. Based on 16S rRNA gene sequence analysis, the iron-oxidizing chemosynthetic bacterium, *Mariprofundus*, seems to make up a substantial proportion of the bacterial community at this interface. *Mariprofundus* likely plays a role in the formation of rust-colored flocculent material in the Double Keyhole water column (Fig. 1) and may influence the export of iron from this ecosystem to the Gulf of Mexico. The Double Keyhole KSE discharges groundwater into the Gulf at an average rate of 1.3 m³/s and is potentially a source of considerable fluxes of iron and other nutrients to downstream surface ecosystems. Our results highlight new aspects of how KSEs regulate fluxes of carbon and nutrients from aquifers to the ocean and draw attention to potential consequences of sea level rise and groundwater over-extraction along carbonate coastlines.



Figure 1. A diver floats among rust-colored flocculent material to collect water samples from the Double Keyhole karst subterranean estuary (© Paul Heinerth).

Plant-microorganism interactions within the rhizosphere for heavy metal soil remediation

Dorina Podar^{1,2,3*}, Cristina L. Văcar³, Emanuela D. Tiodar^{3,4}, Mihaela Gălean^{3,4}, Mihai C. Grimm³, Zoltán R. Balázs^{3,4}, Anastasia Abrudan¹, Csaba Timar¹

¹ Department of Molecular Biology and Biotechnology, Faculty of Biology and Geology, Babeş-Bolyai University, 400084 Cluj-Napoca, Romania, dorina.podar@ubbcluj.ro

² "Emil G. Racoviță" Institute, Babeş-Bolyai University, 400084 Cluj-Napoca, Romania;

³ Centre for Systems Biology, Biodiversity and Bioresources (3B), Babeş-Bolyai University, Cluj-Napoca, Romania, 400084 Cluj-Napoca, Romania

⁴ Doctoral School of Integrative Biology, Babeş-Bolyai University, Cluj-Napoca, Romania

Key words: tolerance, phytoremediation, mercury, *Lotus tenuis*, mercuric reductase (*MerA*), *Fusarium oxysporum*

Soil sustains 25% of Earth's biodiversity, supporting all terrestrial life, and mitigates climate change by retaining carbon and water. Yet, over 60% of soil ecosystems in the EU are unhealthy due to erosion, floods, landslides, loss of organic matter, salinization, contamination, compaction, sealing, and loss of biodiversity. Mercury (Hg) pollution is a global threat due to its toxicity, mobility, and persistence. Despite reduced Hg usage, abandoned mines and chlor-alkali plants remain sources of pollution. European legislation prioritizes managing Hg waste to prevent its spread. High-cost engineering technologies are unsuitable for agricultural soils, making phytomanagement with plants and rhizosphere microorganisms a viable alternative.

Our research focuses on i) assessing the metal resistance strategies of native plants colonizing a polymetallic Hg contaminated site and on ii) identifying rhizosphere-associate microorganisms (bacteria and fungi) that can assist plant growth under Hg contamination. Field study was conducted at a Hg-contaminated site, on the premises of a former chlor-alkali plant. Metal concentrations in soil and plant samples assessed by portable X-ray fluorescence spectrometry showed soil median Hg concentration 96-fold higher than the intervention threshold for industrial sites, reflected by an extremely high potential ecological risk index (ERI) of 769600. Additionally, high ERI for Pb was found. Metal analysis of six spontaneous pioneer herbaceous plant species showed high Hg concentrations in both shoots and roots, linked to the high soil Hg levels. The dominant species was *Diplotaxis muralis* and the legume species *Lotus tenuis* were identified as the most promising for growth on highly Hg-contaminated soil. Microbial communities in the rhizosphere of the dominant species *D. muralis* appeared to rely on Hg detoxification mechanisms. Notably, Actinomycetota has a higher relative abundance in merA-positive rhizosphere communities, which also displayed increased ABC transporters.

Cultivable rhizosphere microorganisms, bacteria, and fungi were assessed for their phenotypic resistance to Hg and other metals. Thirty-two heavy metal-resistant fungi were identified, with Ascomycota representatives showing varied heavy metal resistance spectra, particularly towards ecologically risky elements, unlike Mucoromycota representants. Isolates of *Cladosporium* sp., *Didymella glomerata*, *Fusarium oxysporum*, *Phoma costaricensis*, and *Sarocladium kiliense* displayed Hg biosorption capacities ranging from 33.8 to 54.9 mg/g dry weight, with removal efficiencies between 47% and 97%. A *Fusarium oxysporum* isolate exhibited remarkable resistance to Hg, Cu, Pb, and Zn, achieving 62% removal efficiency of Hg²⁺ from aqueous solution and possible involving biovolatilization (22.02%). Bioinformatic analysis suggested the presence of a mercuric reductase similar to the bacterial merA gene, constitutively expressed, indicating a distinct regulation mechanism.

Knowledge of metal resistance in native plants and rhizosphere-associated microorganisms can enhance phytomanagement by selecting species with high mercury tolerance for sustainable remediation of contaminated sites.

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Emerging contaminants in extreme environments: Microplastics monitoring in karst springs of Montenegro

Marko Nikolic^{1*}, Djuradj Milosevic¹, Ana Manovic², Vladimir Pesic², Dragana Asceric¹,
Melita Mustafic¹, Milica Nikolic¹, Dimitrija Savic-Zdravkovic¹

¹ University of Nis, Faculty of Sciences and Mathematics, Nis, Serbia, marko.nikolic2@pmf.edu.rs

² University of Montenegro, Faculty of Science and Mathematics, Podgorica, Montenegro

Key words: microplastics, karst springs, stygobionts

Microplastic pollution in karst systems remains poorly studied, despite the presence of protected species, habitats, and significant water reserves (Panno et al., 2019; Balestra & Bellopede, 2022). To investigate the presence of microplastics in groundwater and their bioavailability to groundwater inhabitants, we conducted an experiment by collecting hypogeic fauna from karst springs in the field using the kick-net method. Sampling occurred after a period of intensive rainfall, as we anticipated that high water levels would bring stygobionts closer to the surface. The targeted springs were covered to allow these organisms to remain in darkness. Our expectations were met, and we collected amphipods of the genus *Niphargus* and the isopod *Proasellus cavaticus* (Fig. 1). We also found and collected their epigeic congeners, including the amphipods *Gammarus* and the isopod *Asellus aquaticus*. These crustaceans belong to the same functional feeding groups (FFGs) and act as shredders in their habitats. Sampling took place at seven localities near Podgorica, the capital of Montenegro. Epigeic and hypogeic fauna were found at all localities except for one, where no hypogeic representatives were detected. One individual from each sample was preserved for DNA barcoding to verify the morphological identification results. Additionally, water and sediment samples were collected from each location for analysis. Microplastic particles were counted and characterized by size, color, and shape through visual identification under a Leica M165 stereo microscope. Microplastics were detected in all examined habitats; in water, concentrations ranged from 46.2 to 122.47 particles per liter, while sediment samples contained between 4 and 92 particles per 50 ml of ZnCl₂ used for density separation. Based on our results, we can conclude that microplastics are present in underground water and pose a threat to ecosystem functioning and its cryptic biodiversity. Various methods for monitoring microplastics in organisms were tested, but an adequate method is still under development.

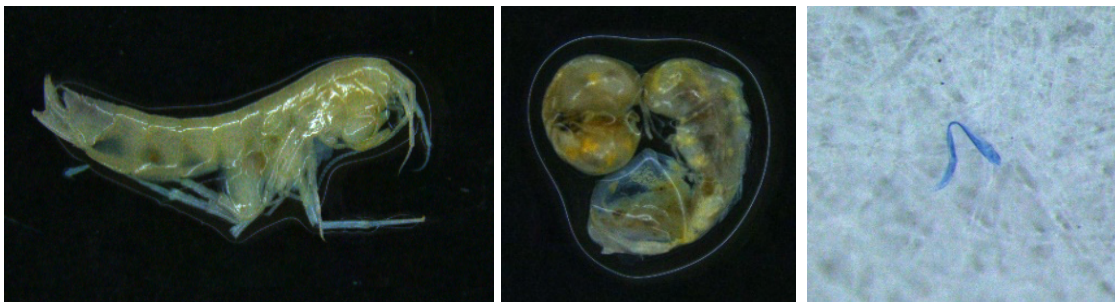


Figure 1. *Niphargus* sp. (left), 2) *Proasellus cavaticus* (center), 3) Microplastic fiber (right).

Acknowledgements: This research is funded by project: Advanced Monitoring of Microplastics in Karst Springs and Groundwater of Montenegro

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Pushing the frontiers on Earth and beyond

Caves across the Solar System

Penelope J. Boston^{1,*}, Michael N. Spilde²

¹ NASA Ames Research Center, California, USA, penelope.j.boston@nasa.gov

² University of New Mexico, Albuquerque, USA

Key words: astrobiology, caves, planetary science, speleogenesis, extremophiles

Finding extraterrestrial caves - What do we currently know about caves on planets and other bodies in our Solar System besides Earth? How can we prepare to detect and study such extreme environments with future space missions? Of course, the place to start this quest is by advancing our knowledge of the vast array of cave types on Earth, how they form, and what mineralogy and biology they contain. Twenty years ago, we developed a physics and chemistry driven agnostic model of speleogenesis that we believe can be applied to Solar System bodies to give us a framework for understanding planetary subsurfaces across the Solar System. We continue to update it in light of new information (Fig. 1; Boston 2004, modified in 2012, 2019, and in Titus et al., 2020).

Earth caves as models - We now know that the subsurface of Earth is a vast biological realm almost unknown to the general public and only seriously accessed by the caving community and a relatively small cadre of scientists through either caves or drilling techniques. Earth caves are semi-closed systems, each one offering a specific set of chemical and physical environmental conditions from hot to cold, wet to dry, containing ordinary air to exotic gas mixtures, and unusual materials such as salt, sulfur, iron, and other metals. Further, we see caves occurring in an array of rock types: lava, limestone, rock salt, gypsum, granite, marble, quartzite, water ice and more. Thus, each cave can be thought of as a distinct miniature model of a “planet” that can help us to understand how geologically dominated biological communities and their mineral environments operate as models for planetary geology and astrobiology.

Vast microbial diversity in the subsurface - The total microbial biodiversity of the Earth’s upper crust to a depth of ~10 km far exceeds that of the surface (e.g., Soares et al., 2023). There are several surprising reasons for this to be the case. The ability to make a living from metabolizing minerals and metals (aka chemolithotrophy) plays a dominant role in the rock fracture habitat on our planet (e.g., Osburn et al 2023) and is also a model for extraterrestrial subsurfaces. The incredible varieties of extremophile organisms in caves, aquifers, and fractures are teaching us many lessons that we are applying to the search for extraterrestrial organisms in future space missions. Other potential homes for life in our Solar System all posit a hypothetical subsurface biosphere including Mars, Europa, Enceladus, possibly Titan and others (Fig. 2).

CAVE TYPE	Dominant Processes	Parent Materials	Earth Examples	Possible Extraterrestrial Variations
Solutional	Dissolving rock by solvent	Soluble solids plus a solvent	Classic karst, gypsum, halite	Non-water solvents, different thermal regimes
Erosional	Mechanical abrasion via wind, water, grinding, etc.	Any solid	Sea coast caves, Aeolian undercuts, etc.	Non-Earth erosional processes, e.g. radiation sputtering, frozen volatiles
Tectonic	Fracturing due to internally or externally caused earth movements	Any rocky solid	Seismic caves	Tidal flexure from a massive primary, crater impact fracturing
Suffosional	Cavity construction by the fluid-borne motion of small particles	Unconsolidated sediments	Mud caves	Ground ice sublimation
Phase Transition	Cavity construction by melting, vaporization, or sublimation	Meltable or sublimable materials capable of solidifying at planet-normal temperatures	Lava tube caves, glacial caves	Perihelionic sublimation of frozen volatiles in comets, frozen bubbles in non-water ices, non-basalt lavatubes
Constructional	Negative space left by incremental biological or accretional processes	Any solid capable of ordered or non-ordered accretion, or biogenic processing	Coraline algae towers, travertine spring mound caves	Crystallization in non-polar ices

Modified from P.J. Boston 2004, Extraterrestrial Caves. In, Encyclopedia of Caves and Karst, J. Goffin, ed.

Figure 1. Physics and chemistry-based cave formation scheme (Boston, 2004-2020).

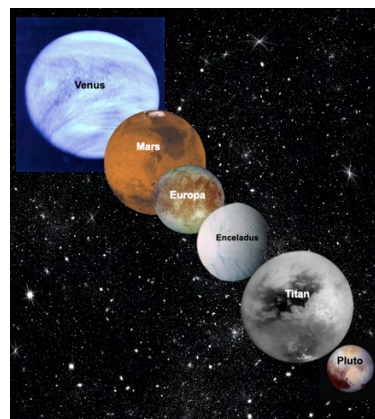


Figure 2. Solar system bodies with caves confirmed or cave potential.

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Environmental information inferred from Mars analogue lava tubes on Earth

Bogdan P. Onac^{1,2*}, Laura Calabrò³, Viorel Atudorei³, Fernando Gázquez-Sánchez⁴,
Francesco Sauro⁵, Ana Z. Miller⁶

¹ School of Geosciences, University of South Florida, Tampa, USA, bonac@usf.edu

² Emil G. Racoviță Institute, Babeş-Bolyai University, Cluj-Napoca, Romania

³ Department of Earth and Planetary Sciences, University of New Mexico, Albuquerque, USA

⁴ Water Resources and Environmental Geology Group, University of Almería, Spain

⁵ Department of Geosciences, University of Padova, Italy

⁶ Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS-CSIC), Seville, Spain

Key words: lava tube, ice, gypsum, stable isotopes, geochronology

Environmental conditions on Mars are difficult to assess, but lava tubes on Earth serve as valuable analogues for Martian tubes and offer insights into them. These underground structures on Earth preserve unique records of geological processes, past climates, and even biological activity, providing a key means of investigating interactions with the planet's surface and atmosphere over time. Studying Earth's lava tubes may enable us to understand their formation and evolution on Mars, as well as how they preserve signs of past environmental conditions, thus offering clues about the planet's habitability and geological history.

In this study, we employ sulfur ($\delta^{34}\text{S}$) and oxygen ($\delta^{18}\text{O}$) stable isotopes to trace the origin of gypsum and reconstruct moisture sources by analyzing samples from El Malpais (USA) and Lanzarote (Spain). Gypsum $\delta^{34}\text{S}$ values indicate that the sulfates in the lava tubes originate from volcanic sources (0–10‰ VCDT) in El Malpais and marine sources (18–24‰ VCDT) in Lanzarote. We inferred that gypsum precipitation was triggered either by sulfates transported from overlying soil by percolating water or by evaporating sea-spray sulfates blown into the tubes. In the former case, $\delta^{18}\text{O}$ values of the ice-melted percolating water are sealed in gypsum's hydration water, allowing the reconstruction of the isotopic composition at the time of mineral precipitation and serving as tracers for hydrological process. The U-series dating further provides deposition timing; for volcanic-origin gypsum, this accurately reflects the lava tube's age, as gypsum forms shortly after lava cooling.

Radiocarbon-based age-depth models for two ice deposits in El Malpais indicate accumulation periods from 7100 to 1600 cal BP in Lava Tube 91 (Fig. 1) and from 3300 to 1000 cal BP in Lava Tube 29. The $\delta^{18}\text{O}$ values in these cores range between –4‰ and –13‰ (VSMOW), suggesting alternating moisture sources from either the Gulf of Mexico and California (with $\delta^{18}\text{O}$ values from –2 to –6‰) or the Pacific Ocean (ranging from –8 to –14‰). A significant shift of 5 to 8‰ towards more negative $\delta^{18}\text{O}$ values, observed around 2900 cal BP in both ice cores, is interpreted as evidence of extended El Niño events, which typically increase winter moisture in the southwestern USA. In contrast, more positive $\delta^{18}\text{O}$ values suggest a strong North American Monsoon (NAM), delivering ^{18}O -enriched summer precipitation. This isotopic pattern marks periods of intense NAM. Additionally, charcoal fragments found in ice layers with intermediate $\delta^{18}\text{O}$ values highlight variable climatic conditions during the ice formation.



Figure 1. Ice deposit in Lava Tube 91, El Malpais, New Mexico (USA).

The most severe drought periods seem to align with La Niña-like conditions and a negative Pacific Decadal Oscillation (PDO), with these droughts becoming more intense when NAM is particularly weak.

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Exploring biosignatures and biogeochemical processes in Lanzarote lava tubes: Implications for Martian analog research

Vera Palma¹, Ana Z. Miller^{2,*}, Francesco Sauro³, Bogdan P. Onac^{4,5}, José M. De la Rosa², Sara Gutiérrez-Patricio², Beatriz Cubero², José A. González-Pérez², Jesús Martínez-Frías⁶, Ana T. Caldeira¹, Nicasio T. Jiménez-Morillo²

¹ HERCULES Lab, University of Évora, Évora, Portugal

² IRNAS-CSIC, Seville, Spain, anamiller@irnas.csic.es

³ Miles Beyond Srl, Padova, Italy.

⁴ School of Geosciences, University of South Florida, Tampa, FL, USA.

⁵ Emil G. Racoviță Institute, Babeş-Bolyai University, Cluj-Napoca, Romania

⁶ Instituto de Geociencias, IGEO (CSIC,UCM), Madrid, Spain

Key words: geomicrobiology, biosignatures, biomarkers, analytical pyrolysis, caves, astrobiology

Lanzarote (Canary Islands, Spain) is a terrestrial analog for Martian volcanology, particularly through its lava tubes, which preserve chemical and morphological biosignatures valuable for astrobiology. Our multidisciplinary study integrates microbiological, mineralogical, and organic geochemistry tools to characterize speleothems and associated microbial communities within these lava tubes. This research aims to understand the factors influencing microbial colonization in Earth's subsurface, and to identify unequivocal biosignatures preserved in lava tubes.

The microbial communities thriving in Lanzarote lava tubes predominantly consist of chemoorganotrophic halophiles and/or halotolerant bacteria, adapted to the surrounding oceanic conditions. These bacteria play a crucial role in reshaping lava tube deposits through carbonatogenic abilities, leaving organic records indicative of past or present life, as revealed by field emission scanning electron microscopy examinations. Based on functional profiling, we infer that *Crossiella* is involved in fluorapatite precipitation via urea hydrolysis and propose biogenic Ca-rich precipitates as compelling biosignatures valuable for astrobiology (Palma et al., 2024a). Analytical pyrolysis, stable isotope analysis, and chemometrics indicate that bacterial communities substantially contribute to the biomarker records in volcanic-hosted speleothems, with lipid fractions primarily comprising low molecular weight *n*-alkanes, α -alkenes, and branched-alkenes.

Molecular, isotopic, and mineralogical characterization of the whitish secondary mineral deposits revealed calcium and sodium sulfates, mainly gypsum and minor thenardite, with sulfur isotopes indicating volcanic and oceanic origins. Carbon isotope compositions and pyrolysis analyses suggest influences from surface vegetation and microbial activity, corroborated by lipidic profiles that highlight microbial origins (Palma et al., 2024b).

Moreover, our detailed analysis of organic biomarkers in gypsum deposits from Timanfaya National Park's lava tube provides insights into the interplay between surface-derived organic matter and in-situ microbial activity. Significant differences in organic matter composition and distribution were noted between surface soil and gypsum layers from the cave environment, indicating contributions from C3 plants and microbial activity. Elemental, isotopic, and chromatographic analyses of a gypsum sedimentary profile reveal varying levels of organic input and microbial reworking, illustrating significant diagenetic changes over time.

This research advances our understanding of environmental dynamics in lava tubes and assists in identifying potential biosignatures both on Earth and other planets. Additionally, our ongoing research in Lanzarote's lava tubes help develop protocols and predictive models for selecting locations and methodologies in the search for potential biosignatures on Mars, with significant implications for astrobiology and the study of subsurface life.

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The environment of glaciovolcanic caves and their value as planetary analogues

Christian Stenner^{1,*}, Lee J. Florea², Linda Sobolewski³, Andreas Pflitsch³, Morgan Cable⁴, Roberto Anitori⁵, Richard E. Davis⁶, Kathleen Graham¹, Glyn Williams-Jones⁷

¹ Alberta Speleological Society, Calgary, Alberta, Canada, cstenner@telus.net

² Washington State Geological Survey, Department of Natural Resources, Olympia, Washington, USA

³ Department of Geography, Ruhr-University Bochum, Bochum, Germany

⁴ NASA Jet Propulsion Laboratory, California Institute of Technology, Pasadena, California, USA

⁵ Department of Biology, Clark College, Vancouver, Washington, USA

⁶ Astromaterials Research and Exploration Science, Texas State University/NASA Johnson Space Centre, Houston, USA

⁷ Centre for Natural Hazards Research, Department of Earth Sciences, Simon Fraser University, BC, Canada

Key words: climatology, geomorphology, glacier, volcano, astrobiology

A unique environment in the cryosphere is generated when thermal flux from volcanic edifices melts void spaces (caves) in ice on volcanoes overlain by glacial ice or firn. Genesis and long-term persistence of glaciovolcanic caves balances the accumulation, ablation, and movement of glacial ice or firn, volcanic heat flux, and the advection of air and volcanic gases through the voids that are formed. Glaciovolcanic caves resulting from these volcano-ice interactions are understudied environments (Sobolewski et al., 2022). However, their geochemistry, microclimates, and morphologies make them useful analogues for planetary environments beyond Earth.

Investigations in glaciovolcanic caves of the Cascade Volcanic Arc, USA, including Mount Hood (Wy'east), Mount Rainier (Tahoma), Mount St. Helens (Loowit), and Mount Meager (Qwelqwelústen) in Canada, reveal their geomorphology, morphodynamics, and microclimate characteristics, along with initial characterization of their subglacial microbial communities. The caves exhibit individually distinct morphological patterns and climate zonation whereby persistent and transient zones exist in a dynamic thermal equilibrium or a lack thereof, dependent on volcanic activity and glacial creep. The environments have zero or low ambient light, are oligotrophic, and have variable ambient air temperatures ranging from slightly below 0°C to 17°C, with subglacial fumarole vents as thermal point sources. Fumarole activity varies from intermittently inactive and 0°C to constantly active and 90°C. Cave sediments have revealed taxonomically distinct microbial communities, some of which may use volcanic and/or trace atmospheric gases for survival.

Climatological and morphological similarities exist between glaciovolcanic caves and conditions on other worlds. High altitude glaciovolcanic caves display relatively low atmospheric pressure and persistent CO₂ accumulations analogous to the Martian atmosphere and lava tube and crevice environments (Stenner et al., 2022). Volcanic gas and steam rising from subglacial conduits to the surface have similarity to cryovolcanism at the South pole of Enceladus, where a global subsurface ocean and possible ocean floor hydrothermal activity reveal favourable conditions for habitability and for future life search missions (Cable et al., 2021). Glaciovolcanic caves are a compelling setting for biogeochemical cycling on other worlds, as life in them may be heavily influenced by the geophysical cycles of ice and hydrothermal centres.

Future research in glaciovolcanic environments to understand properties of thermally influenced icy habitats may provide context for extra planetary exploration and provide insight on geophysical controls on the evolution of extraterrestrial life. Understanding these caves has broader implications to life detection on other worlds, testing robotic mobility and communications, and provides opportunities for analog space mission locations and design.

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Exploring new frontiers in space medicine: unprecedented detailed mapping of human biology during spaceflight

Patras Laura^{1,2,*}, Nadia Houerbi^{3,4}, JangKeun Kim^{3,4}, Eliah G. Overbey^{3,4}, Richa Batra³, Annalise Schweickart^{4,5}, S. Lucotti², Krista A. Ryon³, Deena Najjar³, Cem Meydan^{3,4}, Namita Damle³, Christopher Chin^{3,4}, S. Jan Krumsiek^{4,5}, Jaime Mateus⁶, Xiao Mao, David Lyden^{2,7}, Irina Matei^{2,7}, Christopher E. Mason^{3,4,5,8}

¹ Department of Molecular Biology and Biotechnology, Center of Systems Biology, Biodiversity and Bioresources, Faculty of Biology and Geology, Babes-Bolyai University, Cluj-Napoca, Romania, laura.patras@ubbcluj.ro

² Children's Cancer and Blood Foundation Laboratories, Departments of Pediatrics, Drukier Institute for Children's Health, Weill Cornell Medicine, New York, USA

³ Department of Physiology and Biophysics, Weill Cornell Medicine, New York, USA

⁴ The HRH Prince Alwaleed Bin Talal Bin Abdulaziz Alsaud Institute for Computational Biomedicine, Weill Cornell Medicine, New York, USA

⁵ Tri-Institutional Biology and Medicine program, Weill Cornell Medicine, New York, USA

⁶ Space Exploration Technologies Corporation (SpaceX), Hawthorne, USA

⁷ Meyer Cancer Center, Weill Cornell Medicine, New York, USA

⁸ The Feil Family Brain and Mind Research Institute and WorldQuant Initiative for Quantitative Prediction, Weill Cornell Medicine, New York, USA

Key words: space medicine, space omics, human health, spaceflight, astronauts

Human curiosity and ambition to reach beyond Earth led to an increase in spaceflight missions in the recent years. With the advent of commercial space exploration, the technological developments will allow us to conquer space in the future and to make human life multiplanetary. However, our understanding of spaceflight-induced changes on human biology at the cellular and molecular level is limited. The first all-civilian crew of the SpaceX Inspiration4 (i4) short-term mission (3 days) embarked aboard the Dragon capsule and reached a high altitude (575 km) on the Earth orbit, with the crew being exposed to spaceflight hazards such as radiation, microgravity, and confinement in hostile space environment. The mission provided a platform for mapping human response to acute spaceflight in an unprecedented detail with the largest number of processed biological samples from a space mission, thereby allowing the longitudinal profiling of multi-omic clinical responses across genomics, proteomics, metabolomics, microbiome, and transcriptomics measurements (Jones et al., 2024; Houerbi et al., 2024).

To assess the overall systemic changes using minimally invasive procedures, the plasma proteome (Seer Proteograph), metabolome (LC-MS/MS), whole blood cell transcriptome (Oxford Nanopore), and extracellular vesicles and particles (EVP) proteome were analysed from the i4 crew from blood collected in K2 EDTA tubes at pre-flight (L-92, L-44, L-3) and three post-flight timepoints (R + 1, R + 45, R + 82). EVPs were isolated by sequential ultracentrifugation and were validated via transmission electron microscopy, nanoparticle tracking analysis, Western Blot, and subjected to mass spectrometry analysis (Overbey et al., 2024).

Secretome profiling immediately post-flight (R+1) compared to pre-flight timepoints revealed acute changes in coagulation, immune function, lipid metabolism, oxidative stress, platelet activation, systemic inflammation, and blood-brain barrier function, with some of the dysregulated physiological and stress responses being consistent with other long-duration missions from NASA Twin studies and JAXA. However, most of the changes returned to baseline post-Earth return, thereby suggesting that short-duration spaceflight missions do not pose a significant health risk, but rather allow to measure early phases of the human body adaptation to spaceflight, while expanding the available biomedical database for astronauts and guiding the development of precision astronaut medicine and countermeasures to minimize risks on human health in future missions to the Moon and beyond.

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POSTERS

Preliminary analysis of quartz using scanning electron microscopy techniques

Serban-Constantin Grecu, Alida Timar-Gabor

*Faculty of Environmental Sciences and Engineering, Babeş-Bolyai University, Fantanele 30, 400294, Cluj-Napoca, Romania, serban.grecu@ubbcluj.ro
Interdisciplinary Research Institute on Bio-Nano-Sciences, Babeş-Bolyai University, Treboniu Laurian, 42, 400271, Cluj-Napoca, Romania*

Key words: quartz, scanning electron microscopy, cathodoluminescence, energy-dispersive spectroscopy

Despite being one of the purest minerals, quartz presents minor defects, intrinsic or due to impurities, that might contain important genetic information. Quartz's common occurrence and relative stability during sedimentary processes enables it to preserve chemical signatures, making it an important tool for geochemical analysis and provenance studies (Götze et al., 2021). However, the identification and characterization of these defects is a technical challenge and this information can be unraveled only by conducting a combination of different advanced analytical techniques, such as scanning electron microscopy (SEM) coupled with energy dispersive X-ray spectroscopy and cathodoluminescence (CL) wavelength resolved spectroscopy. In this paper, we present some preliminary results of the application of the specified techniques for different types of quartz grains, extracted from various old to young, fresh versus highly weathered rocks samples. In particular, we performed secondary electron imaging and energy dispersive spectroscopy analysis (EDS) in order to determine sample purity and assess potential contaminants. Afterwards, panchromatic (unfiltered), color filtered, hyperspectral cathodoluminescence imaging, as well as CL spectroscopy were performed in order to determine zonation patterns, textures and contrasts of interest, as well as the main emission bands. The CL emission bands identified have been assigned to different types of intrinsic or extrinsic defects within the quartz structure, as documented in the literature, providing insights into the crystallographic and chemical characteristics of the sample. Two main CL peaks were determined: a 450 nm (2.7 eV) 'blue' emission band, that can be attributed to Ti trace content, according to Götze et al. (2024) and a 650 nm (1.9 eV) 'red' emission band that may correspond to specific intrinsic defects of quartz, with spectral and lifetime characteristics being similar to those of non-bridging oxygen hole centers (NBOHC), according to Skuja et al. (2020).

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The impact of temperature and humidity on the transcriptome of *Symbiochloris reticulata* in relation to *Lobaria pulmonaria*'s genepools

Ioana Ardelean¹, Christoph Scheidegger², Mihai Miclăuș^{3,4}

¹ Biological Research Center, “Babeş-Bolyai” University, Jibou, Romania, ioana.v.ardelean@gmail.com

² Swiss Federal Research Institute WSL, Zürcherstrasse 111, 8903 Birmensdorf, Switzerland

³ NIRDBS, Institute of Biological Research, Cluj-Napoca, Romania

⁴ STAR-UBB, “Babeş-Bolyai” University, Cluj-Napoca, Romania

Key words: Climate change, symbiotic relationships, differentially expressed genes, *Symbiochloris reticulata*, *Lobaria pulmonaria*

The impact of climate change on symbiotic relationships is an emerging field of study. Our research focused on the lichen *Lobaria pulmonaria*, a key indicator species of primeval and conserved beech forests, and a model organism in lichenological studies. We conducted an experiment simulating climate change conditions with varying temperature and humidity in climatic chambers over five days. This included increased temperatures combined with both drought and humid conditions, considering that lichens are poikilohydric organisms, active only in humid state.

Our comprehensive approach involved analyzing all three symbiotic partners: the ascomycetous fungus *L. pulmonaria*, the green alga *Symbiochloris reticulata*, and the cyanobacterium *Nostoc sp.* We aimed to elucidate gene expression changes under stress conditions in two genepools of *L. pulmonaria* from different climatic regions (Continental and Mediterranean) and their impact on the other two symbiotic partners. Our objectives were to identify differentially expressed genes (DEGs) among the three symbiotic partners under these stress scenarios.

Here we present our analysis primarily focused on the green alga to characterize its response to the applied stress factors and its interaction with the two fungal genepools under these conditions. Temperature variations had a more pronounced impact on the number of DEGs compared to humidity variations. Although *S. reticulata* has the same genepool across all our populations, identifying DEGs in samples in symbiosis with the Continental versus the Mediterranean genepool of the ascomycete showed the fungal partner's influence on the green alga's response to heat and humidity stress. Consequently, we speculate that these DEGs play a functional role in the symbiotic interaction. Specifically, in terms of DEGs related to the photosynthetic process of the green alga, we observed a similar response influenced by the fungal symbiotic partner, with the Mediterranean genepool exhibiting fewer DEGs compared to the Continental genepool. This indicates that *S. reticulata* from the Mediterranean region is better adapted to changing climate conditions. Furthermore, although we did not identify DEGs in *Nostoc sp.* in response to heat and humidity treatments, we did find DEGs when comparing samples in symbiosis with the Continental versus the Mediterranean genepools of the ascomycete. This suggests that the cyanobacteria's functioning is significantly influenced by the fungal partner, underscoring its role in the symbiotic relationship.

In conclusion, we identified genes involved in the response of *S. reticulata* to climate change-induced stress. Our findings also highlight the importance of protecting forests harboring the Continental genepool, as they are more vulnerable to the changing climate.



Figure 1. *L. pulmonaria* Mediterranean genepool in a dry and inactive state (left) and in an active state (right).

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Revisiting the chronology of loess in North America by using SAR-OSL and pIRIR protocols

Anca Avram¹, Joseph Mason², Daniela Constantin³, Laura del Valle Villalonga^{3,4},
Serban Grecu^{1,3}, Alida Timar-Gabor^{1,3}

¹ Faculty of Environmental Sciences and Engineering, Babes-Bolyai University, Fantanele 30, 400294
Cluj-Napoca, Romania, anca.giurcea@ubbcluj.ro

² Department of Geography, University of Wisconsin-Madison, 550 North Park ST, Madison, WI, 53706, USA

³ Interdisciplinary Research Institute on Bio-Nano-Sciences, Babes-Bolyai University, TreboniuLaurian
42, 400271 Cluj-Napoca, Romania

⁴ Earth Sciences Research Group, Universitat de les Illes Balears, Ctra. de Valldemossa, km 7.5, 07122
Palma, Spain

Key words: luminescence dating, OSL ages, North America loess

The Loveland loess section, western Iowa is exceptionally well-preserved, providing an invaluable record of past climatic and environmental conditions. The section reaches a thickness of 7 m where the oldest deposit exposed correspond to Marine Isotope stage (MIS) 6. Previous studies on Loveland have reported Optically Stimulate Luminescence (OSL) and Infrared Stimulated Luminescence (IRSL) ages on quartz and polymineral fine grains, respectively (Forman & Pierson 2012; Muhs et al., 2013).

In order to extend the Loveland chronology, OSL dating using three measurement protocols was applied on quartz and polymineral fine grains extracted from seven freshly collected samples. Samples were collected from the same vertical section described in Muhs et al., 2013. Equivalent doses on coarse (63-90 μm) quartz were measured using Single-Aliquot Regenerative Dose (SAR-OSL) protocol while on polymineral fine grains two elevated temperature infrared stimulation methods (pIRIR₂₂₅ and pIRIR₂₉₀) were used. Luminescence ages on coarse quartz range from 49 ± 4 ka obtained on sample LOVE 1 to 76 ± 8 ka on sample LOVE 4. For the older samples, the natural signals were interpolated above the 86% of the saturation threshold. On the other hand, the pIRIR₂₂₅ and pIRIR₂₉₀ ages are in agreement within errors for all samples investigated here, ranging from 33 ± 3 ka on pIRIR₂₂₅ and 39 ± 4 ka on pIRIR₂₉₀ for sample LOVE 1 to 320 ± 45 ka on pIRIR₂₂₅ and 407 ± 51 ka on pIRIR₂₉₀ for sample LOVE 7.

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Luminescence, ESR and CL characterisation of quartz extracted from granite source rocks and the derived sediments used for provenance studies

Daniela Constantin^{1,*}, Aditi Dave¹, Șerban Grecu¹, Zuzanna Kabacińska^{1,2}, Mihai Ducea^{3,4}, Petru Urdea⁵, Alida Timar-Gabor^{1,6}

¹ *Interdisciplinary Research Institute on Bio-Nano-Sciences, Babeș-Bolyai University, Cluj-Napoca, Romania, daniela.constantin@ubbcluj.ro*

² *Faculty of Geographical and Geological Sciences, Adam Mickiewicz University, Poznań, Poland*

³ *Faculty of Geology and Geophysics, University of Bucharest, Bucharest, Romania*

⁴ *Department of Geosciences, University of Arizona, Tucson, Arizona, USA*

⁵ *West University of Timisoara, Department of Geography, Timisoara, Romania*

⁶ *Faculty of Environmental Science and Engineering, Babeș-Bolyai University, Cluj-Napoca, Romania*

Key words: *optically stimulated luminescence, thermoluminescence, electron spin resonance, cathodoluminescence, quartz, granites, sediments*

Quartz luminescence sensitivity as well as some electron spin resonance (ESR) signals have been proposed as potential indicators for the provenance of sediments. While it is largely believed to be acquired by Earth surface processes, recent studies bring empirical evidence that optically stimulated luminescence (OSL) sensitisation processes are a function of source geology or a combination of both.

Here we investigate the luminescence (OSL and TL), ESR and cathodoluminescence (CL) properties of quartz extracted from granite rocks and their related sediments that show different sensitization behaviour. Catalina granite sensitizes in nature and in laboratory while Retezat granite does not. The laboratory sensitization experiment on Catalina samples indicates that repeated exposure to light and irradiation produces an increase in the quartz OSL sensitivity that is significantly lower compared to the natural sensitization achieved in the sediment, suggesting that other factors, yet unknown, during erosion, transport and deposition processes, play a more important role. ESR and CL detected Ti signals only in Catalina granite and sediment while in Retezat sample they lack. Similarly, intermediate temperature peaks (150–220 °C) were visible only in Catalina samples. This confirms previous findings that Ti centres act as electron traps for the thermoluminescence (TL) peaks at 150–220 °C. Thus, we propose that OSL sensitization by multiple cycles of dosing and bleaching is achieved by decreasing the competition to the OSL recombination trap following recombination of electrons originating from the Ti electron trap at intermediate temperatures, in the deep hole trap.

These are promising results that contribute to the development of a quartz fingerprint method that can have a significant impact on quantitative provenance studies.

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Microbial colonization of new lava tubes formed by the Fagradalsfjall Volcano's eruption (Iceland)

Ettore Lopo¹, Francesco Sauro², Ana Z. Miller³, Bogdan P. Onac⁴, Martina Cappelletti^{1,2}

¹ Department of Pharmacy and Biotechnology, University of Bologna, Bologna, Italy, ettore.lopo2@unibo.it

² La Venta Geographic Explorations Association, Treviso, Italy

³ Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS-CSIC), Sevilla, Spain

⁴ School of Geosciences, University of South Florida, 4202 E. Fowler Ave., NES 107, Tampa, FL 33620, USA

Key words: Lava tubes, microbial colonization, microbial communities

The spectacular eruptions of the Fagradalsfjall Volcano (Iceland), which occurred from March to September 2021, resulted in the formation of a 4.8 km² wide lava field (Fig. 1). This unique event provided an exceptional opportunity to monitor and study the microbial colonization of newly formed lava tubes.

With this purpose, every six months, we collected various basalt samples from areas in the surrounding of the eruption sites, and we studied the microbial diversity over time by amplicon sequencing of the community 16S rRNA genes by Illumina MiSeq platform. The same approach was used to study the microbial diversity within the cold, ancient Buri Lava Tube to get insight into the microbial communities' composition of a stable environment that represents advanced stages of the evolutionary process of lava tubes.

The microbial diversity analysis revealed that the initial colonizers were predominantly from the phylum *Pseudomonadota*, accounting for over 98% of the microbial community. After six months, a shift in the microbial community composition was observed, with an increase in the relative abundance of bacteria belonging to the phyla *Acidobacteriota*, *Actinobacteriota*, *Bacteroidota*, *Firmicutes*, and *Chloroflexi*. Although *Pseudomonadota* remained the most abundant phylum, its relative abundance decreased to 70%, and it further decreased within the Buri cave's microbial communities, ranging from 30 to 60%.

The first results of this still ongoing study suggest that members of the phylum *Pseudomonadota* could play a role in the first-colonization event, shaping the substrate and generating the conditions supporting the emergence of the secondary colonizers.



Figure 1. Entrance of one of the lava tubes under analysis (© Vittorio Crobu).

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Assessing extreme environments: is *Diplotaxis muralis* a key species for the phytomanagement of mercury-contaminated soils?

Csaba Timár¹, Maria-Anastasia Abrudan¹, Mihaela Gălean^{1,2}, Dorina Podar^{1,*}

¹ Department of Molecular Biology and Biotechnology and Centre for Systems Biology, Biodiversity and Bioresources (3B), Faculty of Biology and Geology, Babeş-Bolyai University, 400084 Cluj-Napoca, Romania, dorina.podar@ubbcluj.ro

² Doctoral School of Integrative Biology, Babeş-Bolyai University, 400084 Cluj-Napoca, Romania

Key words: mercury resistance, microorganisms, heavy-metals, phytomanagement, ROS-scavenging enzymes

Mercury (Hg) pollution of soils, water and air poses a major threat to both environmental and human health. This is primarily caused by mercury's ability to bioaccumulate and biomagnify within the food chain. Phytomanagement methods, which employ plants and microorganisms, have emerged as environmentally alternatives to industrial approaches for remediating mercury-polluted sites. The present experiment examines the resistance of *Diplotaxis muralis* to various Hg concentrations in conjunction with bacterial and fungal inoculations, to identify their potential use in phytomanagement efforts. Plants were grown for 8 weeks in soils contaminated artificially or anthropically with Hg. Microbial inoculation was performed at 2 and 6 weeks using a bacterium (*Pseudomonas chlororaphis*), a fungus (*Sarocladium kiliense*) or their consortium. Soil metal concentrations were analysed via ICP-OES (Inductively Coupled Plasma – Optical Emission Spectrometry), while chlorophyll, carotenoid and ROS (reactive oxygen species) scavenging enzyme concentration and activity were measured by UV-VIS spectroscopy. Plants cultivated in artificially contaminated soils (22 mg Hg/kg and 55 mg Hg/kg) exhibited significantly higher biomass compared to the control group. Those cultivated in anthropically contaminated soils (285 mg Hg/kg and 759 mg Hg/kg) showed substantially less growth, due to the particularly high Hg concentrations, but overall results emphasise the capability of this plant species to withstand harsh abiotic conditions. Beyond 55 mg Hg/kg, there was a marked reduction in both chlorophyll and carotenoid concentrations, although this was partially alleviated by the fungal inoculum. The inoculation treatments had the greatest effect on the enzymatic activities of catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR), all of which decreased regardless of Hg concentration, suggesting that plant-microbe interactions enhance resistance to Hg. Furthermore, statistical analyses showed that while the inoculation treatments increased resistance to soils with Hg concentrations < 55 mg Hg/kg, higher concentrations of Hg far offset any benefits conferred by microorganisms. These outcomes collectively indicate that *Diplotaxis muralis*, inoculated with the aforementioned microorganisms, is indeed a strong candidate for the phytomanagement of Hg contaminated soils, which have, over time, become extreme environments for living organisms.

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Research in extreme subsurface environments: logistic support and safety considerations

Viorel Traian Lascu^{1,2}

¹ Cave Rescue Romania, 1 Decembrie Square, no. 4-6, 410068, Oradea, Romania, viorel.t.lascu@gmail.com

² National Institute for Research and Development in Environmental Protection, 060031 Bucharest, Romania

Key words: cave rescue, logistic support, safety protocols, specialized training, subsurface environments.

Research in extreme subsurface environments, such as caves and natural voids, presents significant challenges due to harsh conditions including rugged terrain, low temperatures, high humidity, and complete darkness. These environments pose substantial risks to researchers, particularly in archaeological sites, where accessibility can vary due to geological transformations and hazardous conditions.

This study focuses on the essential logistic support and safety measures required for effective research in these demanding settings. It examines the critical equipment needed, including lighting sources, protective gear, and minimal-impact setups, crucial for ensuring the safety of researchers and the integrity of archaeological sites. The study also addresses the need for advanced technological systems and rigorous safety protocols to manage risks and respond to accidents effectively.

Key components of the logistic support and safety include:

1. Risk assessment and planning: Conducting detailed pre-exploration analyses to identify potential hazards and developing intervention plans based on preliminary exploration. This includes selecting equipment that minimizes environmental impact and facilitates access to and research within various types of subsurface environments, including archaeological sites.
2. Specialized training: Ensuring researchers are physically prepared and possess advanced knowledge in speleology, mountaineering, and first aid to handle the extreme conditions encountered in challenging subsurface environments.
3. Cave rescue intervention: Establishing effective response strategies in case of accidents, utilizing specialized rescue equipment and teams trained for extreme conditions.
4. Continuous monitoring and support: Implementing real-time monitoring systems to track researchers and environmental conditions, along with providing ongoing logistic support for equipment and resources.

By integrating these elements, the study aims to provide a comprehensive understanding of the logistical and safety considerations essential for conducting research in extreme subsurface environments, including archaeological sites. This approach enhances researcher safety and ensures the successful exploration and preservation of these unique and challenging environments.



Figure 1. Rescue from below the surface – on the edge of life (© Cave Rescue Romania).

Harnessing the potential of extremophyte sea aster for environmental challenges

Maksymilian Adamczyk¹, Konrad Bąk¹, Anna Rusaczonk¹, Aleksandra Koźmińska²,
Ewa Muszyńska¹, Alina Wiszniewska^{2,*}

¹ Department of Botany, Institute of Biology, Warsaw University of Life Sciences-SGGW, 02-776 Warsaw, Poland

² Department of Botany, Physiology and Plant Protection, University of Agriculture in Kraków, 31-120 Cracow, Poland, a.wiszniewska@urk.edu.pl

Key words: metal toxicity, *Aster tripolium*, halophyte, thiol compounds, abiotic stress tolerance

Among the plants inhabiting extreme environments on Earth are halophytes (salt-loving plants), adapted to growth and development in the presence of excessive salinity. Saline habitats worldwide differ in relation to their ionic composition and origin, but a common characteristic is an excessive concentration of ions in such environment. Since halophytes possess the mechanisms determining tolerance to these unusual conditions, they are capable of maintaining undisturbed growth also in the presence of toxic concentrations of metallic elements. That make these group of plants prospective material to be exploited in environmentally challenging areas, including those heavily contaminated with metals. The sea aster (*Aster tripolium* L., syn. *Tripolium pannonicum* subsp. *Tripolium* (L.) Greuter), a short-lived perennial halophyte growing naturally in the coastal salt marsh areas, has lately gained interest due to its considerable level of metal tolerance (Wiszniewska et al., 2019). Our studies focused on recognizing sea aster responses conditioning tolerance specific abiotic stressors to decipher potential co-tolerance mechanism of this species to multiple environmental stresses.

We have found that, in comparison with reaction to salinity, under metallic stress exerted by Cd²⁺, Cu²⁺, Zn²⁺ and Pb²⁺ ions, sea aster plants employ more advanced osmoprotective and growth-inhibiting strategies, mainly changes in carbohydrate and stress-related amino acids profiles. Growth restriction was attributed to altered phytohormonal regulation, particularly an interplay between salicylic acid and gibberellins, in plants exposed to metals. Considering accumulation of thiol compounds, constituting metal detoxification mechanism, in sea aster we observed metal-specific responses, such as elevated level of free glutathione under Cd treatment in comparison to Cu and Zn, whereas its enhanced binding to proteins (glutathionylation) under Cu. DNA protection system was efficient, as none of tested metals induced fragmentation of genetic material, as well as no changes were detected in relation to oxidative modifications of nucleotides. Tolerance to metallic elements in sea aster was also manifested in low and stable level of lipid peroxidation.

Comprehensive studies covering other aspects of sea aster reaction to metallic stress are currently on the way in our laboratory, including anatomical screening and insight into cell wall rearrangements. Extended stress tolerance of sea aster enhances its perspective applications for desalination, phytoremediation and other activities aiming at combating degradation and pollution of land areas, were unique physiological features of this extremophytic species could be exploited.

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Metallophytes as guardians of highly harsh environments: comprehensive insights into *Silene vulgaris* tolerance to metals

Konrad Bąk¹, Maksymilian Adamczyk¹, Matyjek Jagoda¹, Anna Rusaczonok¹,
Wojciech Borucki¹, Alina Wiszniewska², Ewa Muszyńska^{1,*}

¹ Department of Botany, Institute of Biology, Warsaw University of Life Sciences-SGGW, 02-776 Warsaw, Poland, ewa_muszynska@sggw.edu.pl

² Department of Botany, Physiology and Plant Protection, University of Agriculture in Kraków, 31-120 Cracow, Poland

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Metallic elements are one of the most common non-biodegradable inorganic contaminants in the environment, triggering many harmful ecological changes due to their persistence. However, they can be partially extracted from or immobilized in the place of their occurrence thanks to the natural ability of certain plant species to tolerate enormously high metal content. Such adaptive abilities are observed in representatives of plant communities called metallophytes, which have evolved mechanisms enabling them to survive and repair stress-induced damages. *Silene vulgaris* is one of the facultative metallophytes that appears abundantly in Poland on waste deposits created after mining and processing of lead, zinc and nickel ores. Furthermore, it occurs commonly in unpolluted areas throughout Europe. For this, it is a suitable species for comparative research on specific and conservative responses to toxic metals. Therefore, the overall goal of our experiments is to explain the mechanisms underlying *S. vulgaris* adaptation to thrive in the presence of metallic elements, making this species a potential candidate for phytoremediation efforts. In our interdisciplinary research, special attention is paid to the evaluation of cellular redox status, oxidative damage and the content of enzymatic and non-enzymatic antioxidants as well as ions' accumulation strategies in calamine, serpentine and non-metallicolous *S. vulgaris* ecotypes. The most important achievements suggest that the transformations of reactive oxygen species (ROS) and efficient mechanisms of their neutralization constitute a basis of metal tolerance and lead to proteome remodelling, while the antagonism of Zn and Pb/Cd ions protects against the uncontrolled influx of ballast elements to shoots (Muszyńska et al., 2019, 2020; Muszyńska & Labudda, 2020).

Since ROS seems to play a crucial role in the regulation of *S. vulgaris* reactions to metals, in the presented experiment, we used melatonin as a potential ROS scavenger to determine its effects on plant growth, ROS accumulation, and antioxidant properties under metal stress. Shoot cultures of metallicolous calamine ecotype (M) and non-metallicolous one (NM) were obtained from seeds primed with water (control) or 100 µM melatonin by 24 hr and then multiplied on the medium enriched with the mixture of zinc, lead, and cadmium in the concentration of 714.3 µM ZnSO₄, 3.0 µM Pb(NO₃)₂, and 16.4 µM CdCl₂, respectively. After 8 weeks of metal treatment, the number and length of newly regenerated shoots were measured, and the amount of low-molecular weight antioxidants together with their ability to scavenge DPPH and ABTS radicals as well as Fe²⁺ chelating activity and photosynthetic pigment contents were determined. ROS detection in leaf cells was also performed using a confocal laser-scanning microscope. It was found that metal treatment enhanced ROS accumulation in leaves of both ecotypes obtained from water-imbibed seeds, but only in M leaves their localization was restricted mainly to the palisade parenchyma. In turn, melatonin priming significantly reduced ROS content in NM and M leaves. It also benefitted the growth and fitness of NM cultures cultivated in the presence of metals, whereas the reaction of M cultures was the opposite, and growth disturbances accompanied by increased accumulation of different phenolic compounds occurred. These findings indicate that low ROS level in M tissues causes metal-induced stress despite the evolutionary adaptation of this ecotype to their presence, highlighting the importance of ROS in metal tolerance.

Our experiments collectively advance the field of stress biology by exploring various physiological, biochemical, and molecular responses of the plant to metals. By combining metal detoxification pathways, oxidative stress management and adaptive responses evaluated in contrasting ecotypes, our research provide valuable information for developing phytoremediation strategies and enhancing plant resilience in metal-contaminated environments.

References

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